GHENT UNIVERSITY

ILVO

FACULTY OF VETERINARY MEDICINE
accredited by EAEVE

Marieke DESENDER

ELECTROTRAWLING FOR BROWN SHRIMP

EVALUATING ITS IMPACT ON A SELECTION OF MARINE FISH SPECIES IN THE NORTH SEA

Marieke DESENDER

ELECTROTRAWLING FOR BROWN SHRIMP: EVALUATING ITS IMPACT ON A SELECTION OF MARINE FISH SPECIES IN THE NORTH SEA

Electrotrawling for brown shrimp: evaluating its impact on a selection of marine fish species in the North Sea

Marieke Desender

Electrotrawling for brown shrimp: evaluating its impact on a selection of marine fish species in the North Sea

Marieke Desender

2018

Dissertation submitted in fulfillment of the requirements for the degree of Doctor in Veterinary Sciences (PhD)

Promotors: Prof. dr. Annemie Decostere
Prof. dr. Koen Chiers
Dr. Ir. Hans Polet

Department of Morphology, Faculty of Veterinary Medicine – Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

Department of Pathology, Bacteriology & Poultry Diseases, Faculty of Veterinary Medicine – Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

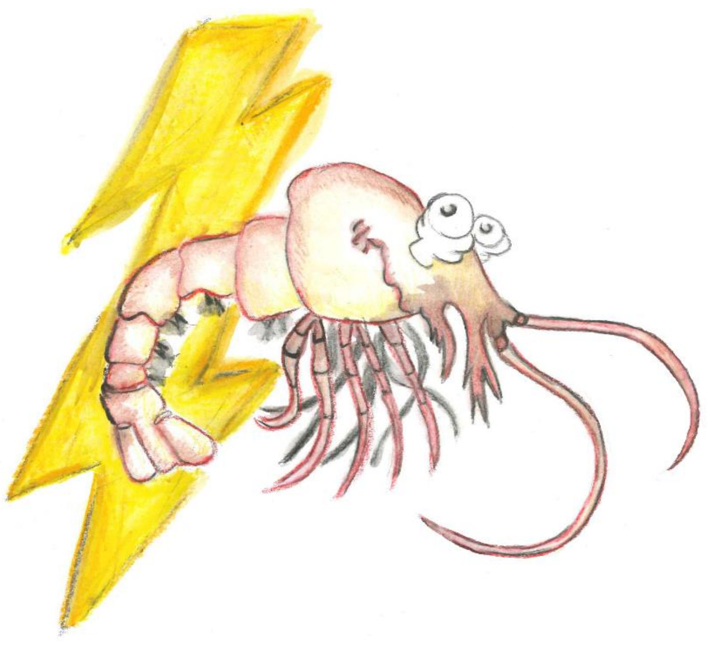
Institute for Agricultural and Fisheries Research (ILVO), Animal Sciences - Fisheries, Ankerstraat 1, 8400 Oostende, Belgium

Cover Design: Jurgen Houben: blueeye.houben@hotmail.com

Electrotrawling for brown shrimp: evaluating its impact on a selection of marine fish species in the North Sea

Marieke Desender

With great “power” comes great responsibility



Contents

List of abbreviations

A	Ampere
AC	Alternating current
AoL	Ampullae of Lorenzini
C	Conductivity
DC	Direct Current
DPE	Days Post Exposure
DPF	Days Post Fertilisation
DPH	Days Post Hatching
Hz	Hertz
ICES	International Council for the Exploration of the Sea
ILVO	Belgian Institute for Agricultural and Fisheries Research
LPUE	Landings Per Unit Effort
MMC	Melano-Macrophage Centers
MSC	Marine Stewardship Council
PDC	Pulsed Direct Current
S	Siemens
TAC	Total Allowable Catch
V	Volt
W	Watt

CHAPTER 1: INTRODUCTION

1 Introduction

Electricity has had a hard time overcoming fear. Still, one could feel the hair stand on end at the thought of electrical devices in the proximity of water. Indeed, according to what most manuals dictate about safe use of electrical appliances, generally water and electricity do not mix. However, electricity has a lot of benefits and is useful in powering everything from the laptop by means of which this text was written to even … fishing gears. With electricity being an essential part to power our daily life, it might be not surprising that developments in fisheries are not standing still either. The last century, electrical fishing (also called electrofishing) or the use of electric fields in water to capture or control fish or other aquatic organisms, has made its way forward.

1.1. Basic Electrical Principles

The effects elicited by electrical fishing are determined by a very dynamic and complex mix of physics and physiology and behaviour (Snyder, 2003). Basically, the generation of an electric field between electrodes in the water and surrounding substrate determines the reaction of the target species. Before discussing electric fishing gear (and its associated electric field), it is necessary to understand some basic principles and terminology used to describe these electric fields.

Every electric charge (measured in coulombs) produces an **electric field**. This may be imagined as a sphere of influence around a charge (Figure 1.1A) in which it will induce a force (attraction or repulsion) on any other charge within the field. Equal positive and negative charges separated with a (small) distance constitute an electric dipole (Figure 1.1B). Electric field strength may be described by the voltage gradient (Volt/cm), the current density (Ampere/cm^2) or power density (Watt/cm^3) (Novotny, 1990). Voltage gradient is most frequently referred to quantify the effectiveness of an electric field, because it may be directly measured in situ (Snyder, 2003; Beaumont et al., 2006).

Voltage or electromotive force is the electrical (potential) energy exchanged when moving a charge within an electric field and is measured in Volt (V=Joule/coulombs). Voltage potential of a particle diminishes with increasing distance from the electrode and may be represented by an equi/isopotential surface or lines of constant voltage in the electric field (Figure 1.1). The difference between electric charge at two points in the electric field will create a voltage/potential difference.

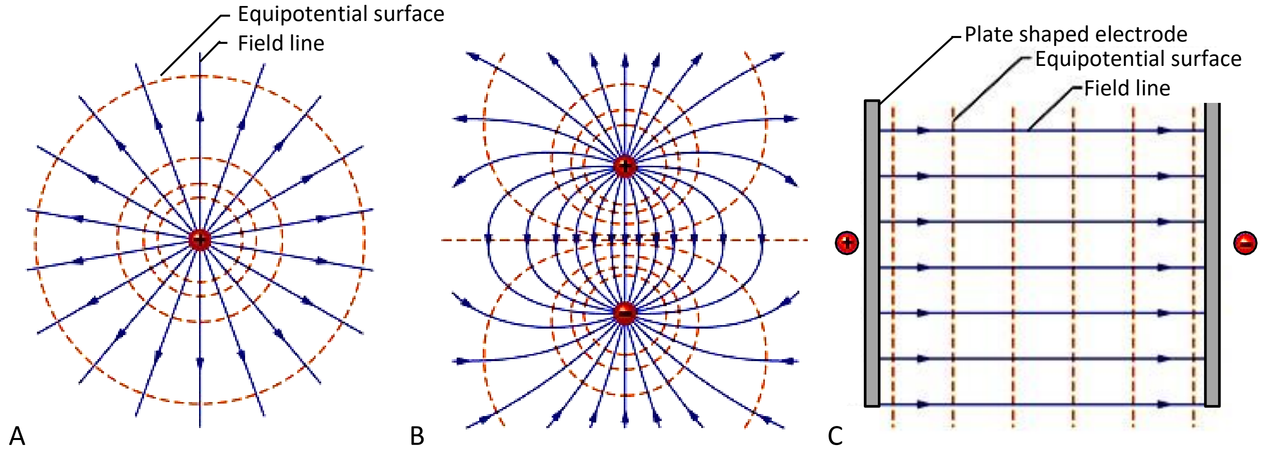


Figure 1.1: Electric fields showing current lines of force (blue solid lines) and equipotential surface lines (red dashed lines) around a A) positive charge B) dipole electric field C) uniform or homogeneous electric field created between two plate shaped electrodes.

Current is the movement of electric charge over a period of time through a cable or any other medium and is measured in amperes (A=coulombs/sec). In the water the electric current is propagated by electrolysis, resulting in a movement of charged ions towards the opposite charged poles. Negatively charged ions are attracted from the negative electrode (cathode) to the positive electrode (anode). However, the conventional direction of current is opposite. Therefore, field lines or (current) lines of force between two charges/electrodes, show the path a positive particle would take as it is forced to move in the field (Figure 1.1) (Morely and Hughes, 1994). Field lines run perpendicular to the equipotential surfaces. Two basic types of current are encountered. When charge carriers flow in one single direction this is called unipolar or Direct Current (DC). When the direction/polarity of current switches, bipolar or Alternating Current (AC) is generated. In electrical fishing DC or pulsed DC (PDC), hybrids between AC and DC, is mostly applied. PDC may be described by frequency (measured in pulses/sec = Hertz (Hz)), shape, pulse duration (expressed in pulse width (µs)) and amplitude (V or A). The waveform amplitude may be described by two voltage measurements: the peak voltage (V_{peak}), maximum voltage, or the root mean square voltage (V_{rms}). The latter is the voltage that would be encountered when the same mean power is applied in DC modus.

Power is the amount of energy expended per unit time or the mathematical product of voltage and current expressed as watts (W=Joules/sec).

The size, shape and distribution of the electric field and its effect on the exposed organism will depend upon many factors including: the form and/or strength of the above mentioned electrical parameters involved such as voltage (gradient); current and waveform generated around the electrodes; the conductivity of the water and surrounding media; the location, orientation, size and shape of the electrodes; basin dimensions and configuration; and the species, its biology/physiology, size and position and orientation between electrodes as these will influence the electric field (Beaumont et al., 2002; Polet, 2010; Breen et al., 2011). The most important variables are discussed below:

Conductivity (of water or aquatic organism), the reciprocal of resistivity, or a material's ability to conduct an electric current, is the most important factor in establishing an electric field. The concentration and nature of charged particles (ions) in the water will determine the water conductivity (Cw). The conductivity of water is measured in siemens (S)/cm. Higher salinity levels in sea water will result in a higher conductivity (53 000µS/cm) in comparison with fresh water (5µS/cm). In high conductivity waters the electric field dissipates easily and thus requires higher power to maintain it. Also, a higher temperature (°C), affecting the mobility of ions, will result in a higher conductivity level. The conductivity of the substrate, depending on porosity and particle material will influence the electric field as well. In high conductivity water, fish have generally a lower conductivity (Cf) than the water. Current is therefore more likely to flow around them instead of through them (Figure 1.2). Consequently, the fish will experience a higher potential difference over its body, between its head and its tail (= head-to-tail voltage).

Electrode design (size, shape, material and placement) will influence the propagation of the electric field. In most natural conditions field lines radiate from a charge/electrode, rendering the electric field non-uniform or **heterogeneous** (Figure 1.1A, B). This means the electric field is stronger close to the electrode where current lines of force are closer to each other. When force lines run in parallel, the field is said to be uniform or **homogeneous**. The electric field strength is constant and may be produced under laboratory conditions using charged plates placed in parallel (Figure 1.1C). In the latter the encountered field strength is therefore not dependent on the location of the fish. However, the highest head-to-tail voltage is established, in both heterogeneous and homogeneous fields, when fish are **orientated** perpendicular between electrodes. Also increased fish/organism **length** will result in a higher head-to-tail voltage. This might be one of the reasons why small fish are harder to catch than large ones. In general, larger fish will show greater reaction and respond to lower field strengths than small fish (McBary, 1956; Stewart, 1975; Emery, 1984; Dalbey et al., 1996; Dolan and Miranda, 2003). However, sensitivity is also **species** dependent (Halsband, 1967).

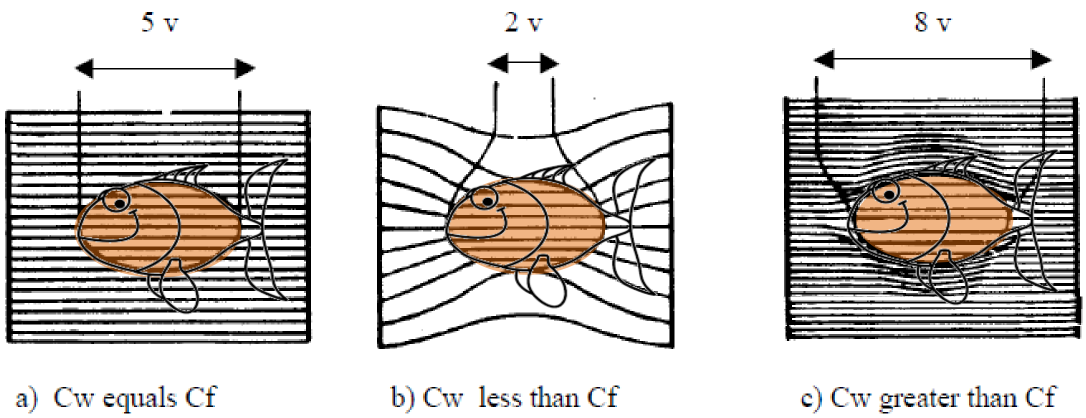


Figure 1.2: Electric field distribution in conductive media: a) similar conductive medium, b) comparable to fresh water, and c) represents the situation in marine water. Horizontal lines represent the current lines of force, the vertical line the head-to-tail equipotential lines (Cw=conductivity of the water; Cf=conductivity of the fish/organism) (Beaumont et al., 2002).

1.2. Electrical fishing: a short outline of its history

Electrical fishing is not new. In the middle of the nineteenth century the first experiments using electricity on animals in water were carried out. Patents granted for electrical fishing date back to 1863 (Vibert, 1967; Snyder, 2003). The initial applications of electrofishing were employed in **fresh water** (Scheminzky, 1924). Nowadays this is a successful and widely distributed sampling technique in rivers, brooks and lakes, mainly used for scientific purposes such as stock assessment, fish health surveys, tagging, catching broodstock and eliminating undesirable species.

The application of electricity in **marine waters** is practically less evident. This is mainly due to the higher conductivity of seawater resulting in less electrical resistance between the electrodes. Therefore, a higher power input is needed in order to maintain a certain electric field. This problem can be overcome by using PDC to save power (Halsband, 1967; D'Agaro and Stravisi, 2009).

The research into applications of using electricity to capture fish at sea started in the 1950s and lasted for several decades. Promising electrofishing techniques were developed, especially in countries around the North Sea (Van Marlen, 1997; De Groot and Boonstra, 1970), the USA (Seidel and Watson, 1978), India (Sreedharan et al., 1977), China (Yu et al., 2007), and the former USSR (Polet, 2010). Only in China a successful commercial fishery using electrobeam trawls to catch shrimp flourished. However, the increase in fishing effort and efficiency together with mismanagement and bad practices by increasing pulse parameters led to a decline in abundancy of the species and consequently a national ban of the method in 2001 (Yu et al., 2007). Electrotrawling was already banned in 1988 in the Netherlands mainly driven by the fear of overfishing due to increase of fishing effort and catch efficiency (Van Marlen, 1997). Also, developments in other European countries ceased around that time. To prevent irresponsible and dangerous fishing practices, unconventional fishing methods, using electricity, explosives, poison or stupefying substances, were **prohibited** in Europe in 1998 (EC nr 850/98 art. 31). However, interest in electrotrawls revived because of increasing fuel costs and the criticism on beam trawls (Kaiser et al., 2006; Polet and Depestele, 2010). Selectivity enhancing practices are encouraged by fishery managers implementing an ecosystem-based approach (FAO, 2009, 2012; Suuronen et al., 2012). Also, the upcoming landing obligation, conservation measures like 'Natura 2000' and a demand from the retail to certify the fishery, put bottom trawling under pressure. Globally, many areas have already been closed for bottom trawling (Polet and Redant, 1999; Polet, 2000; Polet, 2002). More recently there have been claims that the application of electric pulse fields, as an alternative to the traditional mechanical stimulation, could be used responsibly to reduce discards and minimise the benthic impact from demersal fishing gears (ICES, 2010; Polet et al., 2005b; Van Marlen et al., 2014). Electrical fishery in European waters was introduced in the following fisheries during the past years: *Ensis* spp. fishery in Scotland, flatfish and shrimp fishery in the Southern North Sea. Therefore, a **derogation** was manifested in 2009, enabling to equip 5% of each member state's beam trawl fleet with electrotrawls in ICES (International Council for the Exploration of the Sea) zones IVc and IVb south for "scientific purposes" (Figure 1.3) (EU, 2009). The European Commission approved 42 additional licenses for the Dutch fisheries (EU, 2013). This resulted in 84 licenses, amongst which 74 flatfish and 4 brown shrimp cutters, currently active in the Southern North Sea. On 16 January 2018, the European Parliament voted to maintain a ban on catching or harvesting marine species using electricity. Votes will still have to be discussed amongst Member states, the European Council and Commission on the final wording of the legislation (European parliament, Press Releases).

As the main focus of the current PhD research concerns the pulse trawl for brown shrimp, in what follows, emphasis is placed on existing data for this fishery type.

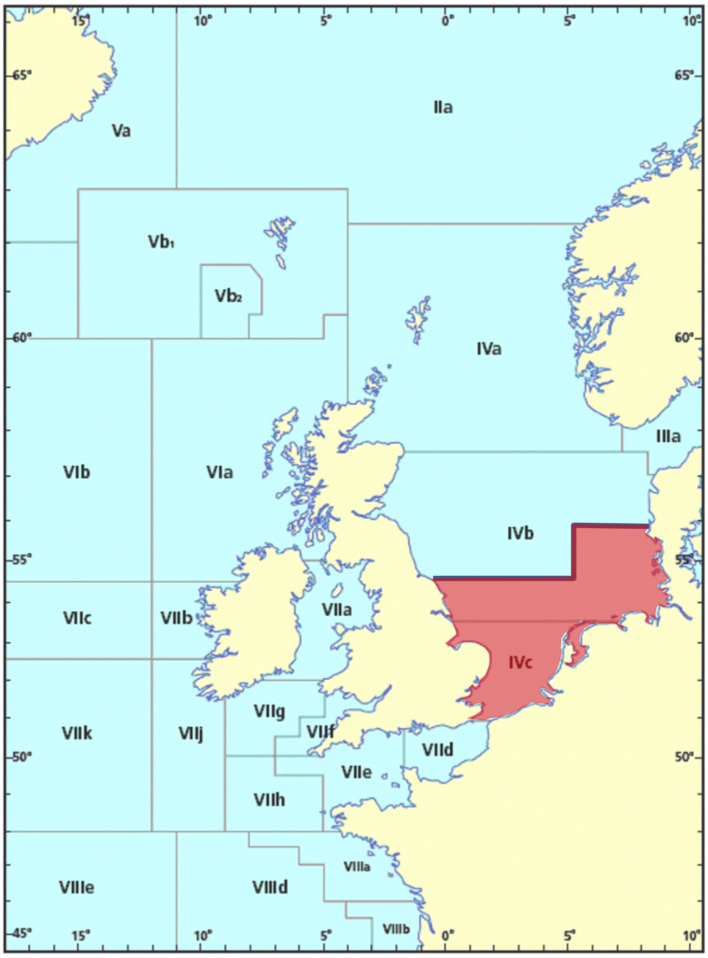


Figure 1.3: ICES rectangles IVc and IVb south allowing electrical fishing.

1.3. Electrotrawling for brown shrimp

1.3.1 Background

The common or brown shrimp, *Crangon crangon*, is an important **benthic key species** (Campos and Van der Veer, 2008; Urzúa and Anger, 2013), since it occurs in high numbers and acts as both a highly efficient predator and important prey (Berghahn, 1996). Therefore, they are an important species in the ecosystem and food web as they can regulate large groups of other species. At night, or during day time when the water is turbid and visibility decreases, it preys on meiofauna and early stages of fish, and bivalves in or on the seabed. During the day, this small crustacean remains half buried with only its antennae protruding to escape from demersal predatory fish, shorebirds and other crustaceans (Hagerman 1970, Campos 2009). This generally highly abundant food source lives along shallow coastal zones and estuaries preferably inhabiting sand and muddy substrates (Tiews, 1970). In wintertime brown shrimp migrate to deeper offshore waters. *Crangon* has a wide **distribution range** extending across the North East Atlantic, as far north as the White Sea of Russia along the European coast till Morocco and is also present in the Baltic, Mediterranean and Black Seas.

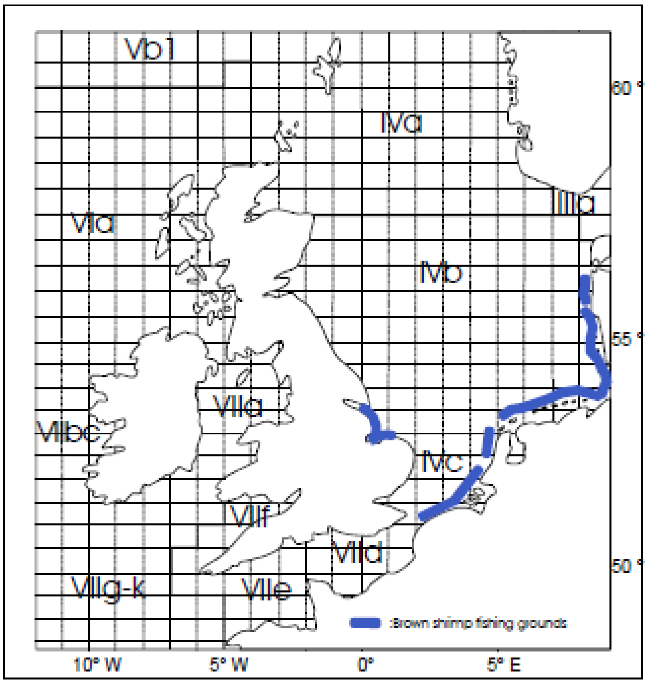


Figure 1.4: Major Brown Shrimp fishing grounds in the North Sea (ICES sub areas Iva, b and c) (Polet, 2003).

Besides being ecologically significant, this species is also economically important supporting a widespread **commercial fishery** with approximately 560 vessels (ICES, 2016), mainly exploited in the coastal zones surrounding the North Sea (Figure 1.4). They produce an annual market value of €70-90 million (Catchpole et al., 2008). This valuable marine resource is among the top three species caught in the southern and central North Sea with respect to landings (ICES, 2012; 2013). The last ten years, total annual landings fluctuated around 35,000 tons, with 37,513 tons in 2014 and 31,375 tons in 2015 (ICES, 2016; Tulp et al., 2016). The fishery for brown shrimp shows a strong seasonal pattern with a peak in catches during autumn. The Netherlands and Germany, together harbouring circa 400 vessels, are responsible for 52% and 37% of all landings, respectively. Fifty-four UK, 44 French, 29 Belgian and 27 Danish shrimpers contribute to the rest of the landing share (ICES, 2015). Belgium accounts for only a small part, with landings reduced from 1,162 tons in 2014 to 670 tons in 2015.

This bottom dwelling species is caught by demersal trawlers. Most vessels are small and have a maximum engine power of 221kW or 300hp to operate inshore (Polet, 2002). Larger vessels, so called Eurocutters, are more common in the Netherlands. The most commonly used gear is a **beam trawl**, generally rigged for twin beaming (Revill et al., 1999). A 6-9 m steel beam supported with trawl shoes on both sides keeps the net open (Figure 1.5). The lower part of the net is attached to a U-shaped ground rope equipped with 24 to 40 bobbins (average diameter: 20cm; average width: 13cm) (Verschueren, 2012). With an average speed of 2.75 knots and an average beam length of 7.65 m, a typical fishing vessel of this fleet will fish a surface of 0.07 km² in one fishing hour (Polet, 2003). The towed bobbin rope startles the shrimp into the net. Subsequently the catch accumulates in the cod-end with a minimum allowed mesh size of 16 mm. After on average 1.5 hours hauling, the catch is sorted on deck by means of a rotating riddle (Polet, 2003; Campos and Van Der Veer, 2008). Subsequently the commercial shrimp fraction is cooked on board changing the shrimp's sandy brown cryptic coloration into their tasteful orange-pink appearance (Vervaele and Fockedey, 2012).

However, **negative effects** accompany this fishing technique which are inherent to the equipment used (Verschueren, 2012). This delicacy is captured along densely populated estuaries and shallow coastal zones such as the Wadden Sea, important nurseries and spawning areas for various marine organisms. Furthermore, the fine cod end meshes, in general 16-20 mm, have poor selectivity and produce high bycatch levels which are often returned dead to the sea (Berghahn et al., 1992; Van Marlen et al., 1997;

Revill et al., 1999; Polet, 2003). At the moment catches contain about 30% shrimp of commercial size, 30% fish bycatch and 30% undersized shrimps (ICES, 2015). In particular the discarding of 0-1-yearold flatfish, such as plaice, may cause a marked negative effect on the fish stocks (Berghahn and Purps, 1998; Revill et al., 1999; Neudecker and Damm, 2010). The plaice bycatch of the Dutch brown shrimp fleet indirectly influences plaice spawning stock biomass with 12–17% (ICES, 2015). In addition, the rather light ground gear (400kg) in comparison with flatfish beam trawl, might impact vulnerable habitats, reducing the diversity of benthic species and changing the functioning of marine ecosystems (Bergman and Hup, 1992; Kaiser et al., 2000; Paschen et al., 2000; Fonteyne and Polet; 2002; Piet et al., 2000; Last et al., 2012).

To avoid these negative effects and hence contribute to the sustainability of this fishery, restrictions on engine power in certain areas, mesh size regulations and various **technical modifications** including bycatch reduction devices, sorting grids and sieve nets were developed (Polet, 2000, 2002; Graham, 2003; Revill and Holst, 2004; Catchpole et al., 2008). Most measures concentrate on the net part of the trawl (Walsh et al., 2000). However, species get injured or stressed during the catch and release process. Preventing unwanted animals from entering the net would hence be a better approach.

Polet et al. (2005a) revealed that brown shrimp selectively reacts strongly to **electric pulses**. A 5-6Hz pulse with a field strength of 240mV/cm (24V/m) was found sufficient to invoke a rapid body flexion escape mechanism, further referred to as tail-flip response (Neil and Ansell, 1995; Arnott et al., 1998), in random directions from all shrimp in each size class and orientation on the bottom. Larger animals showed stronger reactions because of the higher potential difference over their body (Maksimov et al., 1987). Also higher water temperature and darker light conditions enhanced the response (Jeffery and Revill, 2002). Also Stappenbeck (2017) illustrated that shrimp jumped highest after 5-6 pulses in 16 °C and after 8 pulses in water of 8 °C. Approximately 60-70% of shrimp jumped above 10cm when 65V pulse amplitude was applied in water of 12°C in dark conditions (Polet et al., 2005a). Furthermore, with the exception of shrimps, dab and one fourth of the exposed sole, none of the other organisms, plaice (*Pleuronectes platessa*), turbot (*Scophthalmus maximus*), ray (*Raya* spp.), armed bullhead (*Myoxocephalus scorpius*), dragonet (*Callionymus* spp.), pogge (*Agonus cataphractus*), rockling (*Ciliata mustela*), starfish (*Asterias rubens*), brittle star (*Ophiura* spp.) and various crab species, left the seafloor. In this way the adopted electric pulse fields mainly affect target species and replace the mechanical stimulation of the bobbin rope, making it at first sight one of the most promising alternatives to improve the selectivity and hereby reduce the ecological impact of the brown shrimp fishery.

Figure 1.5: Traditional shrimp beam trawl (left) with a U-shaped bobbinrope equipped with 37 bobbins and an electrotrawl for brown shrimp (right) with a straight bobbinrope, 11 bobbins, 12 electrodes and 11 strain relievers (Verschueren et al., 2013).

1.3.2 The Hovercran

Based on a Chinese pulse generator (Yu et al., 2000) and the findings of Polet et al. (2005a), the original electrotrawl for shrimping, the so called Hovercran (HOVERing pulse trawl for selective CRANgon fishing) was developed by ILVO, Marelec n.v. and Ghent University (Figure 1.6 and 1.7). The **basic idea** was to create an electric field that selectively induces a startle response (tail-flip) in the shrimp, forcing them to jump into the water column (Polet et al., 2005a). Other benthic organisms remain mainly on the seafloor and can subsequently escape underneath an elevated groundrope without bobbins (Polet et al. 2005b).

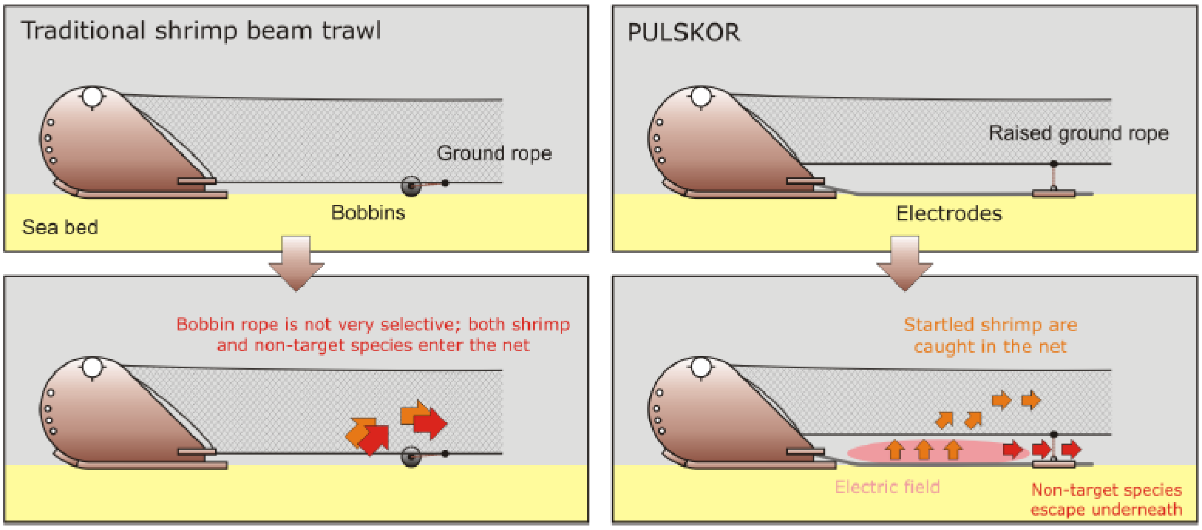


Figure 1.6: Schematic side view illustrating the Hovercran (right) and traditional beam trawl for brown shrimp (left) (Verschueren and Polet, 2009).

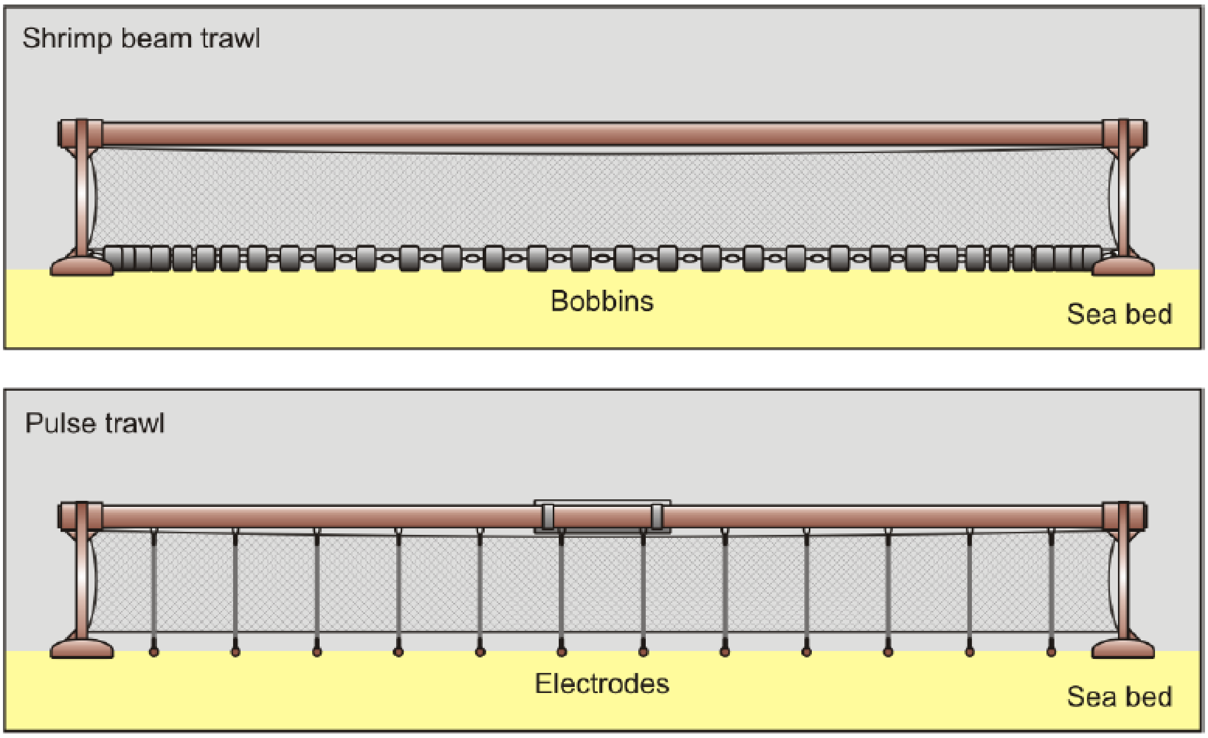


Figure 1.7: Schematic front view illustrating the traditional beam trawl for brown shrimp (above) and Hovercran (below) (Verschueren and Polet, 2009).

Therefore, the **design** of the trawl consists of a pulse generator mounted on an 8m beam. This device converts the AC supplied from the ship to PDC transmitted to 12 lightweight electrodes in the net opening. The electrodes replace the mechanical stimulation by the traditional bobbin rope. They are placed in parallel at a distance of 60 - 70cm. Since no tension is to be applied to the electrodes, strain relievers are attached between the beam and ground rope. One thread-shaped 150cm long conductor has a diameter of 12mm and is composed of six stainless steel strands on the outside and a central solid copper strand inside. The power input is only 1kW per trawl. The maximal amount of current running through the electrodes is circa 250 A_{peak} (12.5 $A_{rms)}$ (Verschueren et al., 2012). To save energy, successive electrode pairs are separately fired with an interval of 20ms. Between two paired electrodes, a half-sine square shaped 5Hz low-frequency, 65 V_{peak}, 23 A_{peak} PDC of 0.25-0.5ms pulse duration is generated every 200ms. Taking into account a trawl speed of approximately 3 knots means a stationary animal is exposed for 1s to the pulse. The created heterogeneous electric field will be approximately 30-50 V m^{-1} in the middle between two adjacent electrodes (Figure 1.8) (Verschueren and Polet 2009; Verschueren et al., 2012).

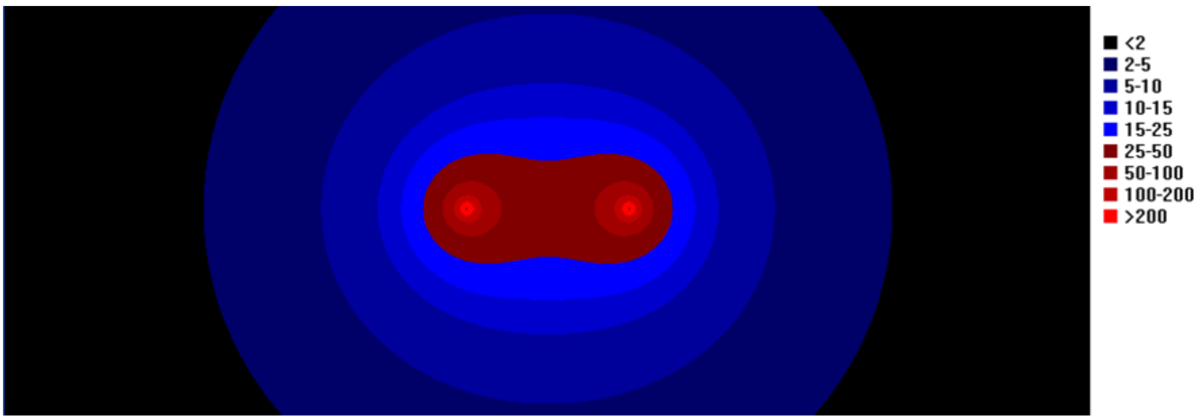


Figure 1.8: Simulation of the electric field strength around a pair of electrodes spaced 60 cm apart. Between two electrodes the electric field strength is approximately 30 V/m (Verschueren and Polet, 2009).

The **reduced environmental impact** of the Hovercran lies herein that there is a reduced seafloor contact up to 76% (Figure 1.9). Furthermore, extensive testing of this device revealed a 35% decrease in discard rate (by volume) while almost equal commercial catches are produced (Verschueren and Polet 2009; Verschueren et al., 2012). Especially the bycatch reduction, probably due to escapes underneath the ground rope, of sole <20 cm and plaice was striking (Verschueren et al., 2012). On the other hand, larger individuals of sole were caught more efficiently indicating a length effect in the reaction of sole to electric pulses. Moreover, catch efficiency of commercial shrimp was less dependent on light conditions and turbidity of the water (Verschueren and Polet; 2009). The lower catch volume in the cod end due to bycatch reduction resulted in pure catches, which improved the apparent quality of shrimp, shortened the sorting process and relieved the workload of the crew. Furthermore, fuel consumption was slightly reduced with 10% because drag resistance is mainly caused by the small mesh size. For the above mentioned reasons, the use of electric pulses in fishing gear is regarded as a promising economically and environmentally friendly fishing method. This very successful system won the 2nd prize in the 2009 WWF Smart Gear Competition. An overview of general pulse and gear characteristics used at sea are given in Table 1.1.

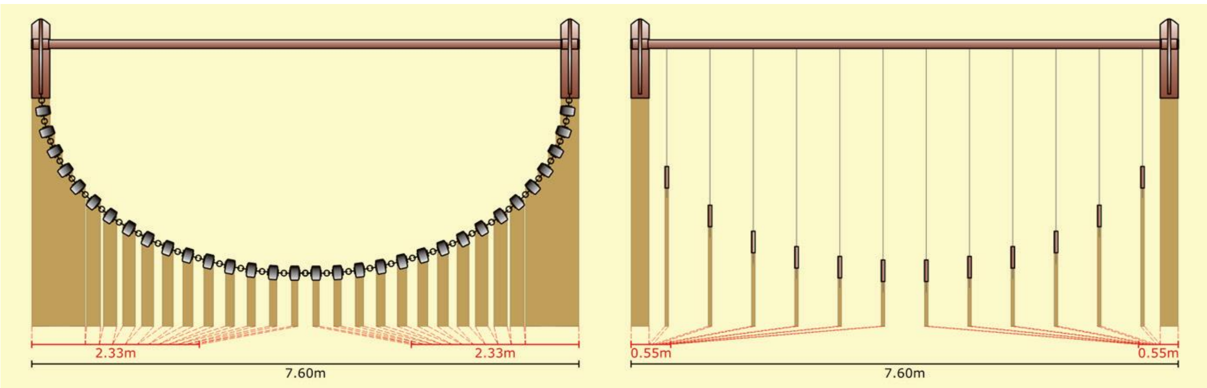


Figure 1.9: Traditional gear with bobbin rope (left), 61% of the seabed is touched by shoes or bobbins. Hovercran (right), 14% of seabed is touched by shoes or electrodes (Verschueren and Polet, 2009).

1.3.3 Variations in design

In the ideal Hovercran vessel, electrodes replace the mechanical stimulation of the bobbin rope, completely. This device is working excellent on smooth seabed surfaces. However, at sea, vessels differ in fishing grounds and consequently rigging, gear configuration and number of bobbins used, (Verschueren and Vanelslander, 2013; Verschueren et al., 2014). Bobbins not only startle shrimp but they also protect the gear on rough and rocky fishing grounds. Therefore, in these areas electrodes are often used in combination with a modified bobbin rope resulting in a diversity of commercial electrotrawls. Dutch pulse shrimpers are enforced to limiting technical measures where a.o. a minimum mutual distance between adjacent bobbins of 60 cm has to be ensured (Pers. Comm. Maarten Soetaert)

When a pulse system is used **together with a traditional trawl**, with U-shaped ground rope, electrodes with varying length, and 36 bobbins, commercial catches may increase up to 50%. However, using this device in commercial conditions is not recommended as wear and tear of the electrodes and unwanted by-catch are very high.

In the search for an alternative gear design that was both economically interesting and environmentally friendly, different innovative designs were explored including variations in nettings, ground rope, weight on electrodes, conductor lengths, … . (Verschueren et al., 2012; 2014; Kratzer 2012; Verschueren and Lenoir, 2016). This quest ultimately resulted in a lighter modified **straight bobbin rope**, instead of a traditional U-shaped bobbin rope, and a **reduced amount of bobbins** (with a minimum of 10) that could be used in combination with the pulse system on commercial vessels (Figure 1.5). In this way all electrodes have the same length, making production and replacement easier. Depending on the rigging, bycatch reductions varied with -15 till -65% (Verschueren et al., 2012). Commercial shrimp volume increased with 13.8%. A sieve net, often used to reduce bycatch of bigger animals in the traditional trawls was not implemented in this set up. With 10 bobbins left on the ground rope, a seabed contact reduction of 50% was achieved. The use of double cod-ends and shortened net opening were considered less interesting due to a.o. higher costs and workload (Verschueren et al., 2012). Also experiments on commercial vessel TH10 equipped with 12 electrodes, a straight bobbin rope (250kg) with 10 rubber discs between 12 bobbins and without an operational sieve net also resulted in improved selectivity (Verschueren and Lenoir, 2016). During September-October 30% more commercial shrimp volume was caught with the pulse system. Also, undersized shrimp volume increased with 63.6%. Additionally, commercial fish species such as plaice, dab and flounder were significantly reduced with 25.9, 30.6 and 71.4%, respectively.

Experiments of the pulse in **combination with a sieve net** resulted in further diminishing of bycaught animals of all lengths, also the smaller individuals. A commercial vessel, HA31, implemented with 12 electrodes, a very light modified straight bobbin rope (155 kg), a sieve net and only 11 elliptic bobbins was monitored in detail from June until December in 2013 at the Dutch Wadden Sea. A lower amount of bycatch, 50-76%, was revealed over the whole season. Especially the reduction of 0-1 year old plaice was remarkable when the pulse was used. Seabed contact was reduced by 50-60% resulting in 23% less drag resistance in comparison with the traditional trawl with sieve net (Verschueren et al., 2014). Furthermore, the catch volume of commercial shrimp was significantly increased during summer with 16%, especially in clear water with low turbidity and during daylight. In contrast, the amount of undersized shrimp was reduced significantly with 19-33% from September till December. Also, in the German electro shrimpers increased shrimp catches and reduced bycatch rates were noted (Kratzer, 2012; Stepputtis et al., 2014).

To conclude, trawl design and rigging may vary considerably between vessels resulting in different outcomes (Table 1.2) considering selectivity and bottom contact (Verschueren and Vanelslander, 2013; Verschueren et al., 2014). Results obtained from commercial vessels cannot be extrapolated to other vessels fishing in different areas. In the field, research is still on-going in close cooperation with the fishing industry to improve this technique, mainly focusing on the reduction of environmental impact. Recently a new system was developed by LFish using a wing-shaped beam covering all electronica, supported by two trawl shoes and implemented with modular electrodes (Pers. comm. Maarten Soetaert). A Dutch novelty is the cable-less 'Jack Wing' wherein the electrical energy is partly generated underwater during towing and stored in battery packs. This makes the use of an electrical supply cable and winch redundant (Pers. comm. Maarten Soetaert).

Table 1.1: Overview flatfish and shrimp pulse system (adapted from Verschueren et al., 2014; Rijnsdorp et al., 2016; de Haan et al., 2016)

Pulse characteristics	**Shrimp**	**Flatfish**
Pulse type	DC, between square and half-sine	Bipolar
Average power supplied per m beam width	0.125 kW	0.6 – 0.7 kW
Maximum conductor voltage$_{peak}$	65 V	45 – 50 V
Pulse frequency	5 Hz	38-42 (Delmeco) 40 – 80 Hz (HFK)
Pulse width (single pulse period)	500 µs	210-230 µs (Delmeco) 100 – 270 µs (HFK)
Duty cycle	0.03%	0.9 – 2.5%
Electrode characteristics		
Number of electrodes	12	10 (Euro cutter) 25 – 28 (>221 kW)
Distance between electrodes	60 – 70 cm	41.5 – 42.5 cm
Total electrode length (isolator + conductor)	2.5 – 3 m	4.75 m
Number of conductor elements	1	6 (Delmeco) 12 (HFK)
Dimensions (length and diameter) of conductor elements	1 (1.5 m x 12 mm)	180 mm x 26 mm (Delmeco) 125 mm x 27-33 mm) (HFK)
Vessel Characteristics		
Width trawl	9 m	4.5 m (Euro cutter) 12 m (>221kw)
Towing speed	2.5 – 3.5 knots	5 knots

Table 1.2: Overview of different modified pulse trawl for brown shrimp configurations and effects regarding environmental impact

Gear	Rigging Pulse trawl	Bobbins (number)	Shape bobbin rope	Control trawl	Shrimp catch	Bycatch	Bottom disturbance	Monitoring	Reference
Hovercran	No sieve net	0	Round	No sieve net	Slight increase	-35% volume	-76%	2008	Verschueren and Polet, 2009
Hovercran (35 different configurations)	No sieve net	0	Round	Control trawl:32 bobbins	Variable	-15 till -65%	-76%	2012	Verschueren et al., 2012
Modified	No sieve net	10	Straight	Control: 36 bobbins Sieve net No sieve net	 +25% +13.8%	 +244% trash -15% trash	-50%		
Modified	Sieve net	11	Straight	Sieve net	+16% June +9% September	-50 to-76% fish and invertebrates whole season -19 to -33% undersized shrimp September till December	-60%	June till December 2013 Dutch Wadden Sea	Verschueren et al., 2014
Modified	Sieve net	11	Straight	Sieve net	+10%	-15% bycatch +14% undersized shrimp		June -August 2012 German Wadden Sea	Kratzer, 2012
Modified					+9%	-9% bycatch +8% undersized shrimp		Extension previous study June- August 2012-2013 German Wadden Sea	Stepputtis et al., 2014
Modified	Not operational sieve net (Combi pulse)	12 + 10 discs	Straight	Not operational sieve net	+30%	-26 to -72% fish +64% undersized shrimp volume		Sept-October Dutch Voor delta	Verschueren and Lenoir, 2016

1.4. Pulse trawling for flatfish

Beam trawls are also used to target flatfish. Flatfish are mechanically stimulated to come out of the seabed with tickler chains or chain matrices instead of a bobbin rope. In general the 4.5-12m flatfish beam trawl is five to six times heavier than shrimp trawling gear. The chains that dig in the seabed till 8 cm (Paschen et al., 2000) are replaced by electrodes that decrease the weight of the trawl and consequently reduce the fuel consumption. In the case of Dutch beam trawlers that switched to pulse trawling for sole, this is approximately 50% (van Marlen et al., 2014). Furthermore, a slower towing speed together with less deep penetration into the sediment by electrodes reduce seabed disturbance (Teal et al., 2014; Depestele et al., 2015). Also bycatch of benthos and undersized fish is reduced by 38 and 56%, respectively (van Marlen et al., 2014; de Haan et al., 2016). Although catch rate of marketable sole was reduced with 21% per hour fishing, additional experiments showed that catch efficiency is actually higher for sole but lower for plaice and other fish species per swept area (Rijnsdorp et al., 2016).

The majority of pulse trawlers, i.e. 74, chase flatfish. The electrical stimulation invokes a cramp response, and U-shaped bending of the body in sole, in the net opening. In this way the fish is immobilized and easier to catch (van Stralen, 2005). To generate a cramped muscle a higher frequency of 45-80Hz is applied. This is the main difference with the shrimp pulse where a low frequency of 5 Hz is created to startle shrimp. Two manufacturers provide pulse gears targeting flatfish, Delmeco (16%) and HFK-Engineering (84%) (Rijnsdorp et al., 2016). The design and pulse characteristics vary by ship and company (Table 1.1). In general a bipolar pulse with a conductor voltage between 45 and 60V and pulse width of 100-270 µs is generated between electrodes of 4.75m (de Haan et al., 2016). Electrodes are spaced approximately 42 cm apart and consist of alternating cylindrical conductors and isolators. The conducting part of an electrode ranges between 26 and 40% (de Haan et al., 2016). Half-way the distance between electrodes, opposite the conducting element, the electric field strength is approximately 75 V m^{-1}.

Fishing vessels <300pk such as euro cutters often switch between shrimp and flatfish as target species depending on the season or market. Therefore, one pulse that could easily combine both types of fishing would be ideal. Although this **combipulse** is technically possible, the heavy pulse components of the flatfish system are unsuccessful to target shrimps (Verschueren and Vanelslander, 2013). Recent experiments indicated a successful integration of the lighter shrimp pulse system and electrodes together with the flatfish pulse system of HFK-engineering (Verschueren and Lenoir, 2016).

1.5. Concerns

As stated above, electrotrawling may be assigned as a promising alternative fishing technique. Nevertheless, the use of electricity in fishing has raised considerable concerns among stakeholders (Kraan et al., 2015). The majority of these concerns are related to possible hitherto unknown deleterious effects on marine organisms and the functioning of the benthic ecosystem. Focus is also set on potential displacement of fishing effort and increased capacity of the fishing fleet and the effects these may have on the sustainable exploitation of the target species and the fishing opportunities of other fleets. The remaining questions are related to governance issues in the framework of economic consequences. In what follows, emphasis will be placed on the **impact** of electric pulses on the target and neighboring aquatic organisms as this is the focus of the present PhD research.

Freshwater electrofishing may inflict harm to fish (Snyder, 2003). The subfamily Salmoninae, trout, salmon and char, seem to be most susceptible. Spinal injuries and associated hemorrhages sometimes have been documented in over 50% of fish. Other harmful effects, such as bleeding at gills or vent, excessive physiological stress, cardiac arrest and mortality by asphyxiation or excessive fatigue are also of concern (Snyder, 2003; Schreer et al., 2004). In addition, electrofishing can affect reproduction and survival or growth of early life stages (Muth and Ruppert, 1997; Henry et al. 2003; Henry and Grizzle, 2004; Bohl et al., 2010). However, reported effects are often contradictory, and are difficult to extrapolate to the marine environment due to differences in conductivity of the water, applied electrical characteristics and variable sensitivity amongst species. Exposure times in freshwater are at least 10 times longer and voltage is often 2-6 times higher (Snyder, 2003).

Research into the effects of exposure of aquatic organisms to electric fields in the marine environment is limited.

1.5.1. Impact on invertebrates

Experiments using the shrimp pulse on **invertebrates** could not reveal major negative effects (Polet et al., 2005a). Only shrimp seemed to react strongly with a tail flip to the low frequency startle pulse. Shore (*Carcinus maenas)* and swimming crab (*Liocarcinus holsatus*) started walking agitatedly on the bottom when stimulated for 15 seconds. No behavioural change was observed in exposed hermit crab (*Bernhardus pagarus*), subtruncate surf clam (*Spisula subtruncata*), common starfish (*Asterias rubens*) and brittle star (*Ophiura* spp.). Survival experiments indicated no mortality amongst shore, swimming and hermit crab and sub truncate surf clam. Survival of brown shrimp was not impaired and ranged between 92.6-107.8% (percentage of animals surviving in the test group compared to the control group) (Polet et al., 2005a). Also, Soetaert et al. (2014) did not find an increase in mortality nor injuries in brown shrimp or ragworm (*Alita virens*) 14 days after exposure to both shrimp and flatfish pulses. During exposure shrimp demonstrated a tail-flip or cramp response depending on the frequency being 5 or 50 Hz (shrimp vs flatfish pulse) whereas ragworm demonstrated a squirming reaction. The survival of shrimp repetitively exposed (20 times in four days) to electric pulses did not significantly differ from those that were repetitively mechanically stimulated. However, the lowest survival was observed for the flatfish pulse, and was significantly lower than in the shrimp exposed to electrodes without electric stimulus displaying the highest survival. No differences were demonstrated for egg loss, food response and the degree of intranuclear bacilliform virus infection (Soetaert et al., 2016c). The latter was in contrast with the previous study, where an increased severity of this infection was observed in the hepatopancreas of shrimp exposed to rather high field strengths of 200V/m Soetaert et al. (2014). The mechanically stimulated shrimp demonstrated the lowest percentage of moults compared to all other treatments, significantly lower than the group exposed to electrodes without electric stimulus in which the highest percentage of moults was noted. In two other exploratory studies, a range of invertebrate species was exposed to the high frequency flatfish pulse. Three to five percent lower survival was found for ragworm and shore crab (*Carcinus maenas*) after two weeks. The latter in addition displayed a 10-13% lower food intake (Smaal and brummelhuls, 2005; van Marlen et al., 2009). Razor clam (*Ensis* spp.) experienced a 7% lower survival only when exposed near the electrode. For the other species such as common prawn, subtruncate surf clam and common starfish no statistically significant effects on survival were found (van Marlen et al., 2009). In search of alternative stimulation mechanisms for other species, the effect on Norway lobster (*Nephrops norvegicus*) was investigated (Stewart, 1972, 1974). Electric pulses could stimulate emergence of Norway lobster from their burrows in less than five seconds. This might increase pressure on egg carrying females.

1.5.2. Impact on fish

Polet et al. (2005a) did not encounter external injuries nor mortality on several fish species, such as sole, plaice, armed bullhead, cod, pogge, dab (*Limanda limanda*), turbot (*Psetta maxima*), dragonet (*Callionymus* spp.), five-beard rockling (*Ciliata mustela*) and gobies (*Pomatoschistus* spp.), as a result of a 15 seconds exposure to the low frequency pulse. Nevertheless, when the pulses adopted in commercial electrotrawling for flatfish were applied, spinal damage occurred in 7-11% of cod and 2% of whiting catches (van Marlen et al., 2014; Rijnsdorp et al., 2016). This effect was also seen in laboratory studies where 40%-70% of marketable sized cod exposed near the electrodes developed a spinal fracture and associated haemorrhages (de Haan et al., 2008; 2016). Smaller fish (12-16 cm) were not affected (de Haan et al., 2011). Other laboratory experiments applying the flatfish pulse on gadoids did not reveal abnormalities following exposure, indicating a highly variable and fish species-specific sensitivity (Soetaert et al., 2016a, b). Also studies performed on sole and seabass did not show increased mortality or injuries 14 days after exposure to the high frequency pulse (Soetaert 2015; Soetaert et al., 2016a). In experiments involving catsharks, no mortality, macroscopic lesions nor aberrant feeding behaviour were observed after being subjected to the flatfish pulse (de Haan et al., 2009). After 9 months the exposed sharks produced eggs. A recent survival study indicated that flatfish pulse trawling showed relatively higher discard survival, overall assessed survival rates were 29% for sole, 15% for plaice and 16% for dab, compared to tickler-chain beam trawl, <10% (van Beek et al., 1990; van der Reijden et al., 2017). Fish were more vital and less impaired in the pulse trawl.

Although the occurrence of **skin ulcerations** in wild fish is not a new phenomenon, an increase in prevalence of 5% was noted since 2011 in dab (*Limanda limanda*) especially during monitoring campaigns in autumn in the Southern bight of the North Sea. Also fishermen reported skin lesions in sole (*Solea solea*) and whiting (*Merlangius merlangus*) in this area (Figure 1.10). Rumours arised that pulse fishing, also introduced in 2011, was involved in the development of skin ulcerations. However, many environmental factors such as an increase in temperature, fluctuations in salinity, algal blooms, skin trauma and viral or bacterial infections might cause or contribute to the development of skin ulcerations. Indeed, a link between seawater temperature and ulcerations was already observed by Mellegaard and Nielsen (1997). Also fishing intensity and physical damage by fishing gear can cause ulceration (Mellegaard and Nielsen 1995; 1997; Wiklund and Bylund 1993; Mellergaard and Bagge 1998).

Figure 1.10: Flatfish delivered by concerned fishermen displaying skin ulcerations (Ghent University).

In May 2012 and April 2014, ulcerated fish were collected from areas subjected to intense pulse fishing and examined for bacteriology and histology. Subacute or chronic skin ulcerations were usually 1-6 cm in diameter and present on both the pigmented and non-pigmented side of the body. Various isolates belonging to different bacterial species were recovered from the ulcerations, but it remained unclear whether these had a causal role or if they were mere an opportunistic colonizer of damaged skin (Figure 1.11).

Previous laboratory exposure tests to electric pulse fields with dab, cod (*Gadus morhua*), and sole (*Solea solea*) did not result in the establishment of skin ulcerations (de Haan et al., 2015; Soetaert et al. 2016a). Recent research has shown that two bacterial pathogens, *Vibrio tapetis* and *Aeromonas salmonicida,* might play a role in the development of skin ulcerations in wild-caught dab (Vercauteren et al., 2016). However, further research is imperative to pinpoint the exact role of these agents in the development of skin ulcerations in common dab and to elucidate if and to what extent pulse fishing may play a role.

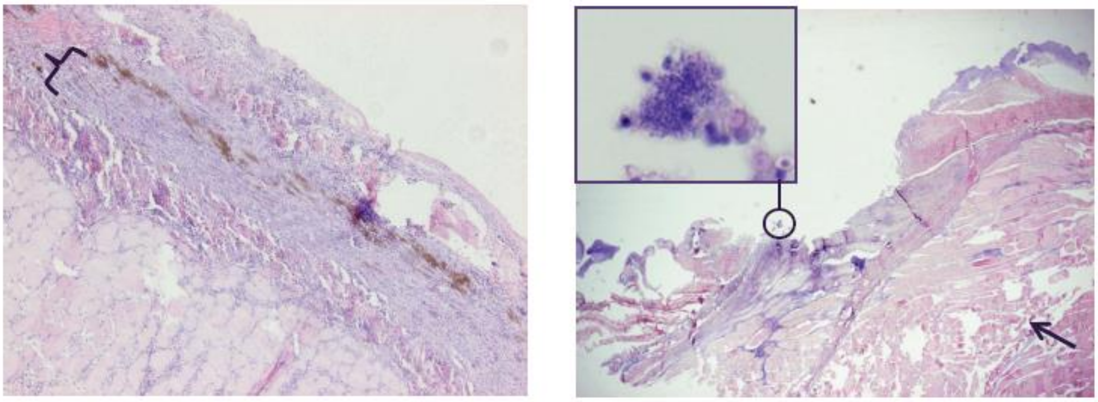


Figure 1.11: Left: Skin of plaice marked fibrosis (accolade) covered with healed epidermis (scar formation) (200x). Right: Skin of plaice: loss of epidermal layer (skin ulceration) (100x). A bacterial microcolony (inset 1000x) is present at the ulcerative surface. The adjacent muscles are degenerated (arrow). HE stain (Ghent University).

A possible impact is not only measured by survival or injuries, it might also cause a **stress response**. Stress stimuli in fish elicit a suite of physiological and behavioral changes. Teleost fish faced with a stressful stimulus launch an endocrine stress response. An immediate adrenaline response prepares the organism for the 'fight or flight' reaction by increasing plasma glucose levels (GC). Shortly after, GCs, in particular cortisol, are released through the activation of a series of endocrine organs, in fish referred to as the hypothalamic-pituitary-interrenal (HPI) axis. These plasma GCs elicit a suite of physiological and behavioral changes that allow the fish to cope with altered situations (Barton, 2002; Øverli et al., 2007). . In this context, they do not have a negative impact on the fish. In contrast, long-term exposure to stressors induces chronic stress leading to detrimental effects including higher susceptibility to disease, compromised functional performance and welfare (Van Weerd and Komen, 1998). In Chondrichthyans such as Elasmobranchs the corticosteroidogenesis is less understood, with 1α-hydroxycorticosterone (1α-OH-B) being the homologue of cortisol (Anderson et al., 2012). To our knowledge, no studies regarding the impact of electrical pulses on the stress levels or response of fish are available.

CHAPTER 2: SCIENTIFIC AIMS

2 Aims

Although electrotrawling seems to be a promising environmentally friendly alternative fishing technique to traditional beam trawling both for brown shrimp and flatfish, very little is known about its possible side effects on target and neighbouring organisms.

Introducing a fishing method based on this technology without a sound knowledge on the interactions between pulse fishing and the marine ecosystem, would be against the principles of the precautionary approach and responsible fishing (FAO, 1995). Further research hence is crucial to re-evaluate electrical fishing and its standing ban in the EU (Council Regulation 850/98). Also the International Council for the Exploration of the Sea (ICES, 2009) recommended more research on the effects of electrical stimuli on marine biota before any larger scale commercial introduction would be considered.

When starting this PhD research, studies into negative effects of exposure to electric fields in the marine environment were limited, resulting in major **gaps in knowledge** with regard to the impact of electric pulses on marine organisms (Quirijns et al., 2015). In addition, most of the experiments were focusing on high frequency electric pulses used in flatfish electrotrawling. Polet et al. (2005a) did not discover injuries nor mortality in fish and invertebrates as a result of subjection to these low frequency pulses. Similarly, no injuries following exposure to low frequency pulses used to chase brown shrimp were found in invertebrates such as shrimp and ragworms (Soetaert et al., 2014; 2016c). However, the former study lacked detailed information on possible macroscopic and microscopic lesions, spinal injury and possible behavioural alterations after exposure. Furthermore, previous studies only included adult fish rendering it impossible to draw well-founded conclusions on the impact of these pulses on young life stages. Additionally, research involving electro sensitive elasmobranchs is very scarce. The research group of de Haan et al., (2009) provided valuable data in that no mortality, external injuries or aberrant feeding behaviour were noted in catsharks after exposure to the flatfish pulse. However, the appetite of the sharks was investigated by using dead fish pieces. Consequently, the effect of exposure to electric pulses on the electro sense organs, the ampullae of Lorenzini, used during the final moments of live prey capture, was not yet investigated when the current PhD was initiated.

The general aim of this PhD research was therefore to provide scientific data to enable the provision of an answer to the question whether electric pulse trawling for brown shrimp is ecologically justifiable by assessing its possible harmful effects on various marine fish species and life stages.

In order to achieve this, experiments were built around three main research questions which were tackled in four chapters:

- What are the short-term effects of exposure to electric pulses used for electrotrawling for brown shrimp in five different marine fish species (European plaice, Dover sole, armed bullhead, bull-rout and Atlantic cod) frequently discarded in the brown shrimp fishery? (Chapter 3)
- What are the effects of exposure of young life stages (embryos, larvae and juveniles) of a selection of marine fish species to the pulse adopted in shrimp electrotrawling? (Chapters 4 and 5)
- What are the effects of electric pulses, used in both flatfish and shrimp electrotrawling, on the functioning of the highly sensitive Ampullae of Lorenzini in small-spotted catsharks? (Chapter 6)

An overview of the **rationale** behind the different experimental set-ups is given in the General discussion p.133situated in the last chapter of the present PhD thesis.

CHAPTER 3: Short-term effect of pulsed direct current on various species of adult fish and its implication in pulse trawling for brown shrimp in the North Sea.

This chapter is based on

Desender, M., Chiers, K., Polet, H., Verschueren, B., Saunders, J., H., Ampe, B., Mortensen, A., Puvanendran, V., and Decostere, A., 2016. Short-term effect of pulsed direct current on various species of adult fish and its implication in pulse trawling for brown shrimp in the North Sea. Fisheries Research, 179: 90-97.

Abstract

Electric pulses in fishing gear are increasingly used in the North Sea and are considered a promising alternative to ameliorate the sustainability of demersal trawl fisheries. The electrotrawl for brown shrimp employing low frequency pulsed direct current (PDC) selectively induces a startle response in shrimp engendering decreased environmental impact and reduced by-catch. Prior to commercially introducing this fishing technique, data on its impact on marine organisms are crucial. The aim of this study was to evaluate the short-term effects of this pulse used for electrotrawling for brown shrimp on five marine fish species inhabiting shrimp fishery areas. For this purpose, 25 European plaice (*Pleuronectes platessa*), 30 Dover sole (*Solea solea*), 20 Atlantic cod (*Gadus morhua*), 19 bull-rout (*Myoxocephalus scorpius*) and 20 armed bullhead (*Agonus cataphractus*) were exposed to the shrimp pulse for 5 s. Before, during and till 20 min following exposure, the behaviour of the fish was monitored. Twenty-four hours post-exposure, all fish were sacrificed, inspected and samples for histological analysis were taken from the gills, dorsal muscle and internal organs. To investigate possible spinal injuries radiographs were taken. Behavioural responses were variable and species dependent. Roundfish species, cod in particular, were displaying more active and agitated fast swimming activity during exposure. The majority of flatfish showed only minor reactions and remained close to the bottom throughout the observation period. However, 15% of the exposed sole actively swam upwards during exposure. Mild multifocal petechial haemorrhages and suffusion, encountered mainly in plaice and sole, were not significantly different between exposed and control groups. Upon histological examination, in two exposed plaice, a focal small haemorrhage between muscle fibers was found, which was not encountered in control animals. In addition, the number of melanomacrophage centres in the spleen of exposed cod was significantly higher than in the non-exposed animals. In conclusion, under the circumstances as adopted in this study, the electric field seemed to have only limited immediate impact on the exposed animals.

Keywords: Electrical fishing, pulse trawling, adult fish, impact, short-term, beam trawl

3.1. Introduction

Brown shrimp, *Crangon crangon*, is an important benthic key species (Campos et al., 2008; Urzúa and Anger, 2013), supporting an important commercial fishery with more than 500 vessels, mainly in the North Sea. This valuable marine resource is among the top three species caught in the southern and central North Sea with respect to landings; 32,277 tons in 2012 (ICES, 2012, 2013). Nevertheless, this delicacy is captured by small demersal trawlers along estuaries and shallow coastal zones of Belgium, the Netherlands, Germany, the United Kingdom, France and Denmark, which are often important nurseries and spawning areas for various marine organisms. Furthermore, the fine codend meshes, in general 20 mm, produce high bycatch levels which are often returned dead to the sea (Berghahn et al., 1992; Van Marlen et al., 1998; Revill et al., 1999; Polet, 2003; Doeksen, 2006). In particular the discarding of 0-1 year old flatfish may cause a marked negative effect on the fish stocks (Berghahn and Purps, 1998; Neudecker and Damm, 2010). In addition, the groundgear might impact vulnerable habitats of the seabed (Kaiser et al., 2000; Paschen et al., 2000; Last et al., 2012). To avoid these negative effects and hence contribute to the sustainability of this fishery, various technical modifications including bycatch reduction devices and sieve nets were developed (Polet, 2000, 2002; Graham, 2003; Revill & Holst, 2004; Catchpole et al., 2008). These practices are encouraged by fishery managers implementing an ecosystem approach to fisheries management and the upcoming landing obligation (FAO, 2009, 2012; Suuronen et al., 2012).

The application of electric pulse fields, as an alternative to mechanical stimulation by tickler chains or a bobbin rope, has proven to be one of the most promising options for reducing potential ecological effect of demersal trawling (Polet et al., 2005b; Van Marlen et al., 2014). Electrotrawls targeting flatfish and/or shrimps were developed since the 1970s (Boonstra and De Groot, 1974; Stewart, 1977) and gained renewed interest from the 1990s onwards (Yu et al., 2007). Although the use of electricity for fishing is still forbidden in Europe since 1988 with Article 3.1 EU regulation 850/98 (EU, 1998), approximately 80 pulse trawlers, among which 5 brown shrimp cutters, are currently active in the southern North Sea (G. Hakvoort, pers. comm.). Indeed, a derogation was manifested to equip 5% of the fleet with electrotrawls in this specific part of the North Sea for scientific purposes (EU, 2009). Recently, the European Commission approved 42 additional licences to Dutch fisheries (EU, 2013). In the original electrotrawl for selective *Crangon* fishing, the so called Hovercran, the bobbin rope is replaced by 12 lightweight electrodes. Between two paired electrodes, a square shaped 60 V low-frequency 5 Hz pulsed direct current (PDC) of 500 µs pulse duration is generated every 200 ms. To save energy, successive electrode pairs are fired separately with an interval of 20 ms. The created electric field selectively induces a startle response in the shrimp, causing them to jump into the net. Consequently, other benthic organisms are left untouched and can escape underneath a hovering trawl (Verschueren & Polet, 2009). Extensive testing of this device revealed 35% to 76% decreases in discard rate (by volume) and a reduction of seabed contact by 50-76% (Verschueren et al., 2012; Verschueren et al., 2014). Furthermore, fuel consumption was reduced and catch efficiency was increased, and fishing is less dependent on light conditions and turbidity of the water. In the field, electrodes are often used in combination with a modified bobbin rope resulting in a diversity of electrotrawls, with the amount of bobbins used and the configuration of the bobbin rope as varying factors (Verschueren and Vanelslander, 2013; Verschueren et al., 2014). Research is still on-going to improve this technique, mainly focusing on the reduction of environmental impact.

Introducing a fishing method on a large scale without a sound knowledge of the interactions between pulse fishing and the marine ecosystem would be against the principles of the precautionary approach and responsible fishing (FAO, 2011). However, the effects of such low frequency pulses on marine organisms are largely unknown (Snyder, 2003; Soetaert et al., 2015). Polet et al. (2005a) assessed the effect of these electric pulses on various demersal fish and invertebrates by taking into account mortality and behaviour during exposure. Although all organisms were still alive 14 d following exposure, the possibly elicited lesions were not evaluated, the behaviour pre- and post-exposure was not studied nor was Atlantic cod (*Gadus morhua*) included in the study. Since spinal injury occurs in 10% of the cod caught on-board of the flatfish pulse trawlers (Rasenberg et al., 2013; Van Marlen et al., 2014), it is imperative to include this fish species in studies evaluating the safety of electric pulses (Soetaert et al., 2016a,b). Snyder (2003) reported that, although often not externally obvious or fatal, spinal injuries and associated haemorrhages may be regularly present as a result of exposure to electricity, warranting the need for radiological and histological examination in safety assessment studies.

It is in this context that the present study was carried out aiming to evaluate the short-term effects of low frequency PDC used for electrical fishing of brown shrimp. This experiment focused on five different marine fish species, frequently discarded in the conventional beam trawl fishery for brown shrimp. These short-term effects were evaluated by assessing behaviour, mortality and presence of macroscopic and microscopic lesions as determined by means of radiological and histological examination.

3.2. Materials and methods

3.2.1 Fish

Adult fish of five different species were selected i.e. European plaice (*Pleuronectes platessa*), Dover sole (*Solea solea*), armed bullhead (*Agonus cataphractus*), bull-rout (*Myoxocephalus scorpius*) and Atlantic cod (*Gadus morhua*). Except for cod, all fish were captured on research vessels RV “Belgica” and RV “Simon Stevin” with an 8 m beam trawl in the North Sea on different sea trips during 2012-2013. Only short (~15 min) fishing hauls were carried out in order to reduce stress and injury caused by the fishing process. On board the fishing vessel, the animals were immediately stored in closed containers with continuous seawater flow. Once ashore, only animals in good condition (no major external injuries, barotrauma, normal movement of opercula, no impaired equilibrium or reaction to stimuli) were transferred to the housing facilities of ILVO in Ostend, Belgium. The different fish species were housed separately in 360 L aquaria, provided with a matured and fully functional biological filter, with a maximum of 10 fish per aquarium. The animals were fed mussel meat and polychaetes (Fonds et al., 1992; Baynes et al., 1993). The water quality was closely monitored and kept at a constant level (14 °C temperature; 34 ‰ salinity; 8 pH; 7.5 dH; <2 5 mg l^{-1} nitrate, <0.2 mg l^{-1} nitrite, <0.1 mg l^{-1} ammonia) and the fish were subjected to a simulated natural photoperiod. All fish were given an adaptation period between 14 and 45 d, during which the general condition and feeding behaviour were observed on a daily basis. Cultured Atlantic cod were obtained from the research facility "Havbruksstasjonen" in Kårvika, Norway. Two months before the onset of the experiment, 40 fish were randomly divided over two tanks of 230 m^3 provided with a continuous flow-through system of seawater connected with the nearby fjord. The cod were continuously fed a commercial diet (Skretting Amber Neptune 300 – 7.0mm) at 0.2-0.4 % of body weight. All experiments were approved beforehand by a Flemish governmental ethical committee (protocol 171/2012) and the Norwegian animal experimental ethical committee (FOTS ID 5183).

3.2.2. Experimental design

After the adaptation period, each fish was individually floy-tagged and transferred to an exposure aquarium (cod: 300 cm x 300 cm x 40 cm; other fish: 600 cm x 140 cm x 50 cm), of which the bottom was covered with rinsed sand (grain size 0.75-1.25mm). The aquarium was equipped with three 150 cm long threadlike electrodes, placed on the bottom of the aquarium. A wire mesh (140 cm x 100 cm x 60 cm) around the electrodes prevented the fish from escaping from the electric field without hampering the field itself. Each electrode had a diameter of 12 mm and was composed of six stainless steel strands on the outside and a central solid copper strand inside. These three conductors were placed in parallel at a distance of 70 cm and were successively electrically connected with a Marelec pulse generator (Marelec Food Technologies, Nieuwpoort, Belgium) representing the last two electrode pairs in the electrotrawl. The other nine electrodes normally used at sea were replaced by electric resistors. Following transfer to the exposure tank, the behaviour of the animals was observed. After 10 min, the generator was manually switched on. During an exposure period of 5 s the same heterogeneous PDC electric field to catch brown shrimp in the field, was generated between each electrode pair in the tank (Verschueren and Polet, 2009; Verschueren et al., 2012). These pulses are characterised by a square pulse shape and pulse duration of 500 µs generated at a frequency of 5 Hz, consequently building up an electric field between each electrode pair with an interval of 200 ms. The applied voltage to the electrodes had a constant amplitude of 60 V. Both electrode pairs were fired separately by the pulse generator with an interval of approximately 20 ms between the first and the second electrode pair. The flow direction of the electric current was constant throughout the exposure. During the experiment pulse characteristics were closely monitored using a Tektronix® Oscilloscope type TDS 1001B. After the exposure, the animal remained for 20 min in the exposure tank before it was returned to the original housing facilities. Twenty-four hours after exposure, the fish was sacrificed by immersion in an overdose of a benzocaine solution (0.1 mg ml^{-1} ethanol) or with MS 222 and examined as described below.

Twenty-four plaice (Length (L): 26.9±3.4 cm), 30 sole (L: 25.5±3.4 cm), 20 armed bullhead (L: 9.7±2.7 cm), 19 bull-rout (L: 19.2±3.2 cm) and 20 Atlantic cod (L: 46.3±3.6 cm) were exposed to electric pulses according to the procedure as mentioned above. For each fish species, control animals were included, namely 25 plaice (L: 28.2±2.8 cm), 20 sole (L: 25.1±3.4 cm), 21 armed bullhead (L: 7.7±2.3 cm), 21 bull-rout (L: 17.0±2.1 cm) and 20 Atlantic cod (L: 46.8±3.0 cm). Length was equally distributed among control and exposed animals. All control animals were treated similarly, except for the exposure to the electric field.

3.2.3. Behavioural analysis, macroscopic, histological and X-ray examination

Each fish was filmed 10 min before, during and 20 min after the exposure to the electric field by means of a Sony camera. The retrieved recordings were analysed focussing on the number of movements (defined as having swum across half the distance of the tank’s length) in the water column or sand before and after exposure, time of first movement after exposure, change in distance and orientation towards the electrodes following exposure. During exposure emphasis was placed on a possible upward reaction, flight response, and cramped or convulsive movements of the body. Following sacrifice, the animals were examined for macroscopic lesions. Special attention was given to possible presence of haemorrhages and discoloured areas on the skin, fins, gills, nares and eyes as these are reported as sensitive for lesions due to electrical exposure (Snyder, 2003). Thereafter, a full necropsy was performed. Samples from gills, heart, liver, spleen, kidney, intestine and dorsal muscle were fixed in phosphate buffered formalin (10 %) for routine paraffin embedding and sectioning. Tissues were dehydrated in graded alcohols and embedded in paraffin wax. Transversal sections of 5 µm thickness were cut with the microtome using the section transfer system (Microm, Prosan, Merelbeke, Belgium). The sections were stained with Haematoxylin/Eosin (HE) and examined with special focus on acute lesions. In addition, the number of melano-macrophage centers (MMC) in the liver, spleen and kidney was counted blindly per section (40x microscopic field). To assess spinal injury, two orthogonal radiographs were taken (50-60 kv, 12.5-16 mAr) from each individual fish, a dorsoventral and a lateral projection, except for flatfish and animals smaller than 7 cm from which only a dorsoventral projection was taken.

3.2.4. Statistical analysis

Statistical analyses were performed separately for each species. A generalized linear mixed model (GLIMMIX procedure in SAS) was fitted to the data using treatment (exposed or control) as a categorical predictor. For counted data, such as number of movements before and after exposure and the amount of MMC’s a Poisson error structure and log-link function were used. Binomial distributions with a logit-link function were used to analyse the change in movement, position and orientation before and after exposure, and for histological and macroscopical data on the presence or absence of particular lesions.

3.2. Results

Neither in the exposed group, nor in the control group mortality was observed for all fish species. In exposed animals, no association was observed between the orientation towards and distance from the electrode and changes in movement, reactions of the body and the macroscopic and histological findings.

3.2.1. Behavioural analysis

For all species included, the number of movements between control and exposed groups was not significantly different within the same species (Table 3.1).

Plaice

Immediately following their release in the exposure tank, all plaice tried to burrow into the sand. Upon exposure to the pulses, the head and tail of all exposed animals simultaneously struck against the sediment for 5 s to the frequency of the pulses. Immediately after the generator was switched off, the flapping stopped. These movements caused one third of the exposed fish to end up more or less deep in the sand during exposure whilst remaining on-site. Two out of the 24 exposed animals displayed a rotation of 90 degrees during exposure, ending up perpendicularly or angled towards the electrode. All fish, except for one control and three exposed animals, maintained their position throughout the full monitoring period.

Sole

After their transfer to the exposure tank, the sole dug into the sand. The control animals remained in the sand during the full observation period. Twenty-one out of the 30 exposed sole showed minor behavioural reactions during exposure. These were limited to slight to moderate flapping movements on-site whilst remaining buried in the sand and retaining their original position except for three fish that were buried deeper in the sand and one individual being fully visible following exposure. Four out of the 30 exposed sole actively swam upward during exposure. Another five exposed fish moved around but remained close to the bottom. Three of these latter animals turned from a perpendicular towards a parallel position. Within one minute post pulse, the animals returned to the bottom where they dug into the sand. Within six minutes after exposure, two of these sole changed position and another three buried themselves deeper in the sand.

Armed bullhead

At rest, the armed bullheads were lying on top of the sandy bottom. Under the influence of the pulses, 11 out of the 20 exposed fish showed rapid jerky movements shooting away close to the bottom. Two fish displayed an upward flight reaction following the onset of the electric pulse, of which one remained vertically positioned along the wall of the exposure tank. After one minute the latter fish returned to the bottom where it resumed normal behaviour and switched its position regularly. Seven exposed fish showed slight vibrations, but did not change their position during exposure. Out of the 13 exposed armed bullhead that changed position, four fish switched from a perpendicular to a parallel position and another three vice versa. During the post-exposure observation period, only five out of the 20 animals remained at the same place. This is in contrast to the control group where 13 animals out of 21 kept their original position.

Table 3.1: Average length of the fish and the number of movements in control (C) as well as exposed (E) animals for each species observed 10 min before and 20 min after exposure.

Species	Number of animals		Length (cm)		10 min before exposure		20 min after exposure	
			Average	Stdev	Average	Stdev	Average	Stdev
Plaice *Pleuronectes platessa*	C	25	28.2	2.8	0.0	0.0	0.0	0.2
	E	24	26.9	3.4	0.0	0.0	0.1	0.3
Sole *Solea solea*	C	20	25.1	3.4	1.7	2.6	0.0	0.0
	E	30	25.5	3.4	0.4	0.9	0.2	0.5
Armed Bullhead *Agonus cataphractus*	C	21	7.7	2.3	2.3	8.8	0.4	0.9
	E	20	9.7	2.7	2.7	5.5	4.4	6.5
Bull-rout *Myoxocephalus scorpius*	C	21	17.0	2.1	2.2	2.3	2.0	2.6
	E	19	19.2	3.2	5.7	10.3	5.5	6.2
Atlantic cod *Gadus morhua*	C	20	46.8	3.0	31.9	10.7	58.4	26.1
	E	20	46.3	3.6	19.5	17.5	36.3	29.0

Bull-rout

All except for three out of the 19 exposed bull-rout laid on the bottom before the pulses started. Indeed, three animals kept on swimming around in the aquarium. When the pulses began, 12 fish started to move around rapidly, close to the bottom, while their bodies vibrated at the frequency of the pulses. Five fish displayed the same jerky movements but additionally swam upward. One fish did not move during exposure and seemed paralysed. Six bull-routs of different sizes and varying position towards the electrodes, showed wide-open opercula and fully spread pectoral fins during exposure. Four animals changed their position from perpendicular to the electrode to parallel and another four vice versa. When the pulses were stopped, the animals resumed their original behaviour within a few minutes. Following exposure, clearly more exposed fish,17 out of the 19, displayed an active behaviour during the 20 min observation period following exposure, whilst for the control animals this was 10 out of the 21.

Atlantic cod

During the settling period, nine of the 20 exposed Atlantic cod displayed hardly any movement, that is less than eight circulations in 10 min, in the exposure tank. The rest of the animals were circulating in the aquarium making full use of the available water space. As soon as the electric field was switched on, all exposed animals started swimming agitatedly in random directions, hereby regularly bumping against the walls of the tank. During the full 5 s of exposure, the fish’s body showed small jerky movements to the frequency of the pulses. Five exposed fish seemed paralysed during the exposure, of which one fish displayed wide-open opercula. After the pulses were switched off, five out of the nine exposed fish that were relatively motionless before exposure and one control fish hardly showed any movement and remained virtually motionless in the corner of the tank for the entire post-exposure observation period. The others resumed their circulating movement in the aquarium within 10 min.

3.2.2. Macroscopic examination

The data resulting from the macroscopic inspection of the animals are listed in Table 3.2.

In plaice, multifocal cutaneous petechiae covering less than 4 cm^2 of the skin near the tail were found in 16 out of the 24 exposed animals and in 14 out of the 23 control organisms, but with no statistically significant differences between them ($F_{1,45}$=0.17; p=0.6815; Figure 3.1 A). Suffusion of the body and fins was likewise noted to a similar extent in control as well as exposed animals. No further abnormalities were encountered.

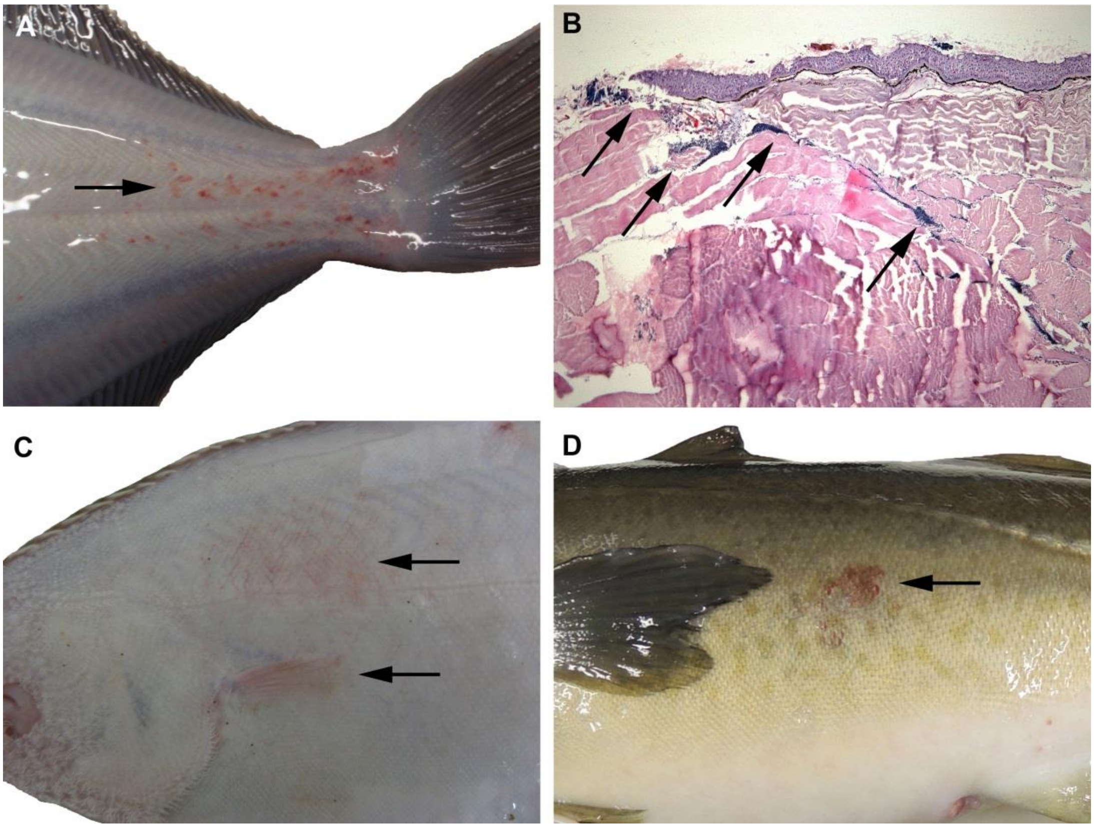


Figure 3.1: Overview of encountered macroscopic and histological lesions in exposed fish: A) Multifocal cutaneous petechiae covering less than 4 cm^2 of the skin nearby the tail in a plaice; B) A small focal interstitial haemorrhage between dorsal muscle fibres in a plaice (H&E staining, 20x magnification); C) Suffusion of the body and pectoral fin in a sole; D) Cutaneous petechiae in an Atlantic cod.

In sole, two out of the 29 exposed animals displayed cutaneous petechiae while one of the control animals also showed this feature; there is no statistical difference between these two groups ($F_{1,47}$=0.07; p= 0.7875). Suffusion of the skin and fins was noted in both exposed (27 and 34 %, respectively) and control (20 and 50 %, respectively) animals, resulting in no significant differences ($F_{1,47}$=0.37; p=0.5483 and $F_{1,47}$=1.17; p=0.2853; Figure 3.1 C).

Furthermore very minor suffusion of the pectoral fins was observed in two out of 20 exposed and three out of 20 control armed bullhead ($F_{1,36}$=0.12; p=0.7262). One exposed and one control cod showed small cutaneous petechiae (Figure 3.1 D). No macroscopic abnormalities were detected in any of the bull-rout, neither in the control, nor in the exposed animals.

Table 3.2: Overview of encountered macroscopic and histological lesions in control (C) and exposed (E) fish.

Species		Plaice		Sole		Armed bullhead		Bull-rout		Atlantic cod	
		Pleuronectes platessa		*Solea solea*		*Agonus cataphractus*		*Myoxocephalu s scorpius*		*Gadus morhua*	
Treatment		C	E	C	E	C	E	C	E	C	E
Number of fish included		23	24	20	29	20	20	21	19	20	20
Macroscopic examination											
Petechiae	Body	61%	67%	5%	7%	0%	0%	0%	0%	5%	5%
Suffusion	Body	30%	29%	20%	27%	0%	0%	0%	0%	0%	0%
Suffusion	Fins	87%	79%	50%	34%	15%	11%	0%	0%	0%	0%
Histology											
Dorsal Muscle	Bleeding	0%	8%	0%	0%	0%	0%	0%	0%	0%	0%
	Vacuolar degeneration	8%	8%	0%	0%	0%	0%	0%	0%	0%	0%
Gills	Acute lesions	46%	42%	53%	50%	26%	35%	47%	50%	25%	25%

3.2.3. Radiographic and histological examination

Upon inspecting the radiographs, no abnormalities were observed.

The histological findings are listed in Table 3.2. In two exposed plaice a small focal interstitial haemorrhage between muscle fibres was found (Figure 3.1 B), which was not encountered in any of the control animals. Focal vacuolar degeneration of the muscle was noted in two exposed and one control plaice. In the gills, acute anomalies including telangiectasia of the secondary lamellae, initial fusion of lamellae and lamellar oedema were noted. These were present in 26 to 53 % of the fish of all tested species captured at sea, both in control and exposed animals, with no significant differences in occurrence between these two groups (all p>0.55). The number of MMC in the spleen was significantly higher in exposed cod compared to the non-exposed animals ($F_{1,37}$=9.26; p=0.0043). The number of MMC in liver and kidney samples was not significantly higher in exposed animals of any of the species included (all p>0.43) (Table 3.3). Chronic gill lesions, that is mild to moderate hyperplasia and fusion of the lamellae, or a bacterial epitheliocystis infection were observed in 23 % of both control and exposed individuals. Intestinal submucosal granulomas mostly associated with encapsulated parasites were found in six exposed plaice and one control bull-rout. In one exposed bull-rout, the liver contained an amorphous light reflecting deposit; in another control sole renal granulomas were observed. No further abnormalities were found.

Table 3.3: The number of melano-macrophage centers (MMC) in liver, spleen and kidney in control (C) and exposed (E) animals. The number of MMC in the spleen of exposed cod was significantly higher compared to the control animals (* p=0.0043)

Species	Treatment		Liver		Spleen		Kidney	
			Average	Stdev	Average	Stdev	Average	Stdev
Plaice	C	23	0.0	0.0	5.0	2.0	6.5	2.0
Pleuronectes platessa	E	24	0.1	0.3	3.9	2.0	5.7	1.7
Sole	C	20	1.1	1.1	2.4	1.5	1.6	1.1
Solea solea	E	29	1.0	0.9	2.3	1.1	1.2	1.2
Armed bullhead	C	20	0.0	0.0	5.9	4.3	0.1	0.3
Agonus cataphractus	E	20	0.0	0.0	4.5	4.7	0.0	0.0
Bull-rout	C	21	0.0	0.0	3.8	1.4	0.2	0.6
Myoxocephalus scorpius	E	19	0.0	0.0	3.6	2.0	0.4	1.0
Atlantic cod	C	20	0.0	0.0	0.5*	2.2	0.0	0.0
Gadus morhua	E	20	0.0	0.0	1.5*	3.6	0.0	0.0

3.3. Discussion

The vast majority of studies on the harmful effects of electrical fishing focus on its use in freshwater, wherein electricity is widely adopted as a sampling technique for fishery ecology and management purposes (Snyder, 2003; Polet, 2010). Information regarding the adverse effects of electrical fishing on marine organisms hitherto is scarce. In the present study, no mortality was observed which corresponds to the findings of Polet et al. (2005a). In freshwater fish, spinal injuries and associated haemorrhages have sometimes been reported (Snyder, 2003; Schreer et al., 2004). The principal cause of spinal injuries appears to be powerful convulsions of the body musculature induced by sudden changes in the electric potential. These sudden changes occur when the current is switched on and off or pulsed. In PDC, longer exposures subject the fish to more pulses and thereby increase the risk for spinal injury, with the incidence of injuries being lowest for low frequency (≤ 30 Hz) PDC (Snyder, 2003). Besides minimizing frequency, results from several studies suggest that the field strengths should also be kept to a minimum to limit injuries (Shreer et al., 2004). In the present study low-frequency PDC, electric square-shaped pulses of 60 V with 500 µs pulse width during 5 s were generated successively between each electrode/resistor pair. These pulse characteristics did not result in marked macroscopic lesions. Suffusion and the small petechiae on the skin in the tail region that were equally encountered in exposed and control plaice, most likely were the result of capturing the fish and releasing them in the exposure tank with the animals fluttering in the sand. This can also be the case for the suffusion and small petechiae encountered in sole. No histological lesions were noted, except for a microhaemorrhage of the dorsal muscle in two exposed plaice. These microhaemorrhages were not found in control animals, hence pointing towards the exposure as the eliciting factor. Bleeding at gills or vent, as reported in previous studies on freshwater fish (Snyder, 2003) were not noted.

In addition to spinal damage and haemorrhagia, other negative effects reported in fish due to electroshock include behavioural disturbances (Shreer et al., 2004). In general, fright, electrotaxis and electronarcosis are three basic reactions that may be noted. Many different variables such as electrical output, electrode size and shape, environmental conditions and species dependent biological factors determine the ultimate reaction inside the electric field (Snyder 2003; Sternin et al., 1972; Sharber & Black, 1999). In the present study, behaviour before, during and after the exposure to the electric field was recorded. A range of behavioural effects during the exposure was observed depending on the fish species. Overall, the flatfish species plaice and sole appeared less responsive to the pulses with reactions that can be denominated as minor for the vast majority of exposed sole remaining close to the bottom following exposure. Fifteen percent of the exposed sole actively swam upward during exposure. This number is less than the 25 % observed in experiments performed by Polet et al (2005a), but remains promising for exploration towards a startle pulse for catching sole.

Nowadays the flatfish pulse trawls, which have quite different characteristics compared to the shrimp pulse trawls, use a bipolar cramp pulse of 40 to 80 Hz causing a cramp reaction of the sole enabling them to be shovelled up from the seafloor. However, damaged cod were caught in commercial trails using the flatfish cramp pulse (Rasenberg et al., 2013; Van Marlen 2014). During experiments performed by De Haan et al. (2008, 2011; 2016), spinal injury in cod exposed to the flatfish cramp pulse was observed in one quarter of the animals when stimulated close to the electrode. When exposed in a homogeneous electric field, in one of the 39 cod a spinal column dislocation occurred (Soetaert et al., 2016a,b). The finding in the present study that spinal injury was not encountered in cod, underscores the opportunity for developing and employing a startle pulse substituting the cramp pulse for flatfish leaving cod unscathed. Cod was the most reactive fish species responding very actively to the pulses and swimming agitatedly in random directions. The way the bull-rout and armed bullhead responded to the exposure varied depending on the individual, with fish not responding or being immobile and animals agitatedly swimming around. Stewart (1975) showed that fish reactions to electric fields are theoretically length dependent with lower effects for smaller fish. The bigger sizes of cod in comparison with the small sizes of bull-rout and armed bullhead could explain the less active behaviour of the last two bottom dwelling species. Overall, the brief fright reactions could be considered as minor effects.

In the current experimental setup, the animals were not fixed in a net so as to ensure a specific distance and orientation towards the electrodes. Not fixing the animals during exposure allowed to fully observe their behaviour during the administration of electric pulses as it was not restricted by a net. In exposed animals, no association was observed between the orientation towards and distance from the electrode and changes in movement, reactions of the body and the macroscopic and histological findings. However, a drawback of this protocol is that the number of animals per position in the electric field is too small to discern a significant effect in all the various behavioural outcomes according to the animal's orientation to and distance from the electrodes and the possibility to correlate these to the induction of lesions and changes in occurrence of MMC.

A significantly higher number of MMC was encountered in the spleen of exposed cod. MMC are reported to increase in size or frequency in conditions of environmental stress, hence serving as biomarkers for environmental quality (Agius and Roberts, 2003). Whether the MMC in the spleen of cod are triggered by the administration of the electric pulses and the possible responsible mechanism(s) remain to be elucidated and warrant further research. In the other species involved in the current study, this phenomenon was not observed.

A mild to moderate epithelial hyperplasia of the gill lamellae and consequent fusion of the secondary lamellae, as well as teleangiectic lamellae and lamellar oedema were noted in all tested fish species that were captured in the wild, regardless of their being exposed or not. Epithelial hyperplasia with fusion of adjacent lamellae constitutes a chronic response of the gill tissue towards a variety of possibly etiological agents (Speare and Ferguson, 2006). In view of the chronic nature of these anomalies and the fact that the animals were sacrificed 24h following exposure, these cannot be attributed to the exposure and are inherent to working with animals that are captured in the wild. Borucinska and Frasca (2002) found gill hyperplasia in 20 % of free-living healthy smooth dogfish (*Mustelus canis*). A considerable amount of these sampled smooth dogfish also displayed hepatic and renal lesions. Stentiford et al. (2010) found grossly visible liver neoplasms in up to 18 % of dab (*Limanda limanda*) populations sampled at some UK marine sites. In the present study, one sole also displayed renal granulomas. Gill anomalies including telangiectasia and oedema can be easily reproduced as artefacts due to euthanasia (Ferguson et al., 2013). In the current study, these features are therefore most likely coincidental since they occurred in fish whether or not exposed to the electric field.

The exposure assays were confined to clinically healthy adults. Electrofishing over active spawning grounds may significantly affect survival of embryos that are present on or in the substrate if they are exposed during their more sensitive stages of development (Polet, 2010). Exposure of recently hatched larvae might reduce growth rates, induce malformations or worse, cause mortality. The exposure of near-ripe or ripe broodstock fish to electric fields may also hamper natural reproduction (Snyder, 2003). The effect on juveniles hitherto is not sufficiently known. Future studies hence are needed in which different life stages of the various fish species are included in order to fully grasp the impact of electrofishing throughout their lifecycle.

In conclusion, the present study demonstrates that, under the circumstances as adopted in this experimental set-up, electric pulses used for catching brown shrimp seem to have only limited immediate impact on the exposed adult plaice, sole, armed bullhead, bull-rout and cod. Although these findings still substantiate a tentatively but cautiously positive attitude towards electrical fishing in terms of sustainability, various major gaps of knowledge still remain and hence need to be investigated to identify and possibly mitigate potentially harmful effects of electrical stimuli on marine biota. These include but are not limited to the impact on various life stages and long-term and indirect effects.

Acknowledgements

This research was supported by a PhD grant from the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT-Vlaanderen). Additional support was given by the Flemish Scientific Institute for Agricultural and Fisheries Research (ILVO), Aquaculture infrastructures for excellence in European fish research (AQUAEXCEL) and the European Fisheries Fund (EFF). This research was facilitated by Flanders Marine Institute (VLIZ) and the crew of the RV "Simon Stevin" and RV "Belgica". We thank David Vuylsteke for his assistance in taking care of the animals and Christian Puttevils and Delphine Ameye for embedding and processing the samples.

CHAPTER 4: Impact of pulsed direct current on embryos, larvae and young juveniles Atlantic cod (*Gadus morhua*) and its implication on electrotrawling for brown shrimp.

This chapter is based on

Desender, M., Decostere, A., Adriaens, D., Duchateau, L., Mortensen, A., Polet, H., Puvanendran, V., Verschueren, B. and Chiers, K. 2017. Impact of pulsed direct current on embryonated eggs, larvae and young juveniles of Atlantic cod (*Gadus morhua*) and its implication in electrotrawling for brown shrimp. Marine and Coastal Fisheries 9; 330-340

Abstract

The application of electric pulses in fishing gear is considered a promising option to increase the sustainability of demersal trawl fisheries. In the electrotrawl fishery for brown shrimp, an electric field selectively induces a startle response in shrimp. Other benthic organisms remain mainly on the seafloor and escape underneath a hovering trawl. Previous experiments indicated that this pulse has no short-term major harmful effect on adult fish and invertebrates. However, the impact on young marine life stages is still unknown. As brown shrimp are caught in shallow coastal zones and estuaries, important nurseries or spawning areas for a wide range of marine species, electrotrawling on these grounds could therefore harm embryos, larvae and juveniles.

In this study, experiments were carried out on different developmental stages of cod (*Gadus morhua*) which are considered vulnerable to electric pulses. Three embryonic, four larval and one juvenile stage were exposed to a homogeneous electric field of 150 V/m_{peak} for 5 s mimicking a worst case scenario. No significant differences in embryo mortality rate were found between control and exposed groups. However, in the embryonic stage exposed at 18 days post fertilization (DPF), the initial hatching rate was lower. Larvae exposed at 2 and 26 days post hatching (DPH), exhibited a higher mortality rate than the corresponding non-exposed groups. In the other larval and juvenile stages, no short-term impact of exposure on survival was observed. Morphometric analysis of larvae and juveniles revealed no differences in measurements or deformations of the yolk, notochord, eye and head. Although exposure to a worst case electric field did not impact the survival or development of six out of eight young life stages of cod, the observed delayed hatching rate and decreased survival for larvae might indicate an impact of electric pulses and warrants further research.

Keywords: Pulse fishing, life stages, survival, development, cod, morphometrics

4.1. Introduction

Beam trawls are used extensively in the North Sea to catch brown shrimp (*Crangon crangon* L.) and flatfish, in particular sole (*Solea solea* L.) and plaice (*Pleuronectes platessa* L.) (STECF, 2014). However, this demersal fishing technique negatively impacts the marine environment with high by-catch rates, intense bottom contact and high fuel consumption as major drawbacks (Jennings and Kaiser, 1998; Lindeboom and de Groot, 1998; Paschen et al., 2000). In order to deal with the upcoming discard ban and contribute to a more ecosystem-based approach in fisheries management (FAO 2009; 2012), one should strive toward measures mitigating the disadvantages of traditional beam trawling by reducing seabed contact and enhancing selective fishing (Polet, 2002; Revill and Holst, 2004; Catchpole et al., 2008). Electrical pulse fishing offers a promising alternative technique meeting these requirements (Boonstra and De Groot, 1974; Polet et al., 2005a; Soetaert et al., 2015). Using these devices, the mechanical stimulation in the ground gear by tickler chains, chain matrices or bobbins is (partly) replaced by electrodes providing electric pulses.

The electrotrawl targeting brown shrimp uses a 5 Hz low-frequency pulsed direct current (PDC) of 0.5 ms creating a field strength of minimum 30 V/m between two thread-shaped electrodes placed in parallel at a distance of 60 cm. In this way, a startle response (tail-flip) is selectively induced in the shrimp, forcing them to rise into the water column (Polet et al., 2005a). Other benthic organisms remain mainly on the seafloor and can subsequently escape underneath an elevated groundrope (Polet et al., 2005b). Therefore, in the original electrotrawl for brown shrimp, the so called Hovercran, all 36 bobbins attached to the ground rope of the conventional trawl, used to mechanically startle the shrimp and protect the ground gear, are removed (Verschueren and Polet, 2009; Verschueren et al., 2012). However, at sea, vessels differ in rigging, gear configuration and number of bobbins used, resulting in different outcomes regarding selectivity and bottom contact (Verschueren and Vanelslander, 2013; Verschueren et al., 2014). To exemplify this, a commercial electrotrawl for brown shrimp was monitored in the Dutch Wadden Sea in 2013. However, also 11 bobbins were implemented on a modified straight bobbin rope, instead of 36 bobbins and a traditional U-shaped bobbin rope used in a traditional gear. A 76% decrease in discard amount and a 60% reduction of seabed contact resulting in 23% less drag resistance were noted (Verschueren et al., 2014). Furthermore, the catch volume of commercial shrimp was increased in summer with 16%, especially in clear water with low turbidity and during daylight. For the above mentioned reasons, the use of electric pulses in fishing gear is regarded as a promising fishing method both from an economic and environmental point of view.

Fishing with electricity in the sea has been prohibited since 1998 in Europe (EU, 1998). In 2009, an exemption was granted allowing each member state to equip 5% of its beam trawl fleet with electric pulse gears in the southern part of the North Sea (EU, 2009). In 2013, 42 additional licences were allocated to Dutch fisheries (EU, 2013). In view of the rapid expansion of electrotrawling, there is an urgent need to improve our knowledge on possible adverse effects of these pulses (Yu et al., 2007; Quirijns et al., 2015). Introducing a fishing method based on this technology without a sound knowledge on the interactions between pulse fishing and both target and non-target marine organisms would violate principles of responsible fishing (FAO, 2011).

Previously, in short-term laboratory conditions, the electric fields used in the shrimp pulse fishery seemed to have a limited impact on exposed adults of targeted or bycaught organisms (Polet et al., 2005a; Desender et al., 2016, 2017a; Soetaert et al., 2014). Still, the potential impact on young life stages is a growing concern that has not been addressed.

As electrofishing is a commonly used sampling technique in rivers, ponds and lakes, research on the impact of electric currents on eggs, larvae and juveniles previously focused on freshwater species. Indeed, electric fields could negatively affect young organisms, with intensity and type of electric field, exposure duration, developmental stage and species as determining parameters (Snyder 2003). However, one should keep in mind that data resulting from the use of electric currents applied in fresh water with different electrical settings cannot necessarily be extrapolated to the marine environment due to differences in conductivity. Field intensity used in freshwater is 2-6 times higher and duration 10-60 times longer than in seawater. As these are the most critical parameters affecting embryos and larvae (Dwyer et al., 1993; Dwyer and Erdahl, 1995), it might be assumed that effects would be more moderate in seawater than freshwater (Soetaert et al., 2015). Whether the latter hypothesis is correct needed to be empirically investigated. Indeed, studies addressing the effects of electrofishing on young life-stages of marine species are not available. These data nevertheless are crucial as brown shrimp are often caught in shallow coastal zones adjacent to extensive tidal flat areas such as the Wadden Sea that are often important nurseries and spawning areas for a wide range of marine species.

The current study is the first to expose Atlantic cod (*Gadus morhua* L.) at various embryonic, larval and young juvenile stages to electric pulses targeting brown shrimp and to evaluate their survival. Exposure might not cause a significant increase in mortality but may reduce growth rates for at least a few weeks (Muth and Rupert, 1997). Therefore, morphometric analysis was performed at two chosen time points for each developmental stage. Atlantic cod was adopted as a model organism for marine cold water round fish species. This commercially important top predator is considered vulnerable to high-frequency electric pulses as observed in commercial catches on board flatfish pulse trawlers (Rasenbergh et al., 2013; Van Marlen et al., 2014) and during laboratory experiments performed by de Haan et al. (2009; 2011; 2016).

4.2. Materials and methods

4.2.1. Experimental animals

Fertilized eggs from strip-spawned captive broodstock of three different spawning events, batches, maintained at the Centre for Marine Aquaculture Research (NOFIMA, Tromsø, Norway) were incubated until the desired developmental stage. When approximately 50% of hatching occurred, this was referred to as 0 DPH (days post hatching) in larval age. In total eight developmental stages were exposed, resulting in eight experiments, ranging from early cleavage in the embryonic stages (batch 1), to larval stages (batch 2) to juveniles following metamorphosis (batch 3). An overview of the experiments are illustrated in Table 4.1. Three stages of embryos at 1, 5 and 18 DPF (days post fertilization) (experiment 1-3), four larval stages at 2, 11, 26 and 46 DPH (experiment 4-7) and one young juvenile stage at 60 DPH (experiment 8) were exposed to electric pulses as described below. Each experiment was performed in triplicate. Three control groups were also included, of which the fish were treated in exactly the same way as the exposed animals except for the fact that the electric field was not switched on.

After exposure, survival was counted until 2DPH every five days for exposed embryos, one week past exposure for exposed larvae and 29 days following exposure for juveniles. Also morphometric characteristics were measured (Figure 4.1). Samples for morphometric analysis were taken at two time points for each experiment. The first time point was at 2DPH for embryos and one day following exposure for larvae and juveniles. The second time point was at least 15 days and maximum 31 days following exposure.

All experiments were approved by the Norwegian animal experimental ethical committee (FOTS ID 5185).

Table 4.1: Overview of the different experiments across three batches and eight developmental stages of Atlantic cod showing when samples were taken for exposure to electric current, survival and morphometric analysis. DPF (days post fertilisation), DPH (days post hatching). *50% of embryos hatched at 21 DPF = 0 DPH.

Experiment	Batch	Developmental stage	Exposure	Survival	1st morphometric sample point	2nd morphometric sample point
1	1	Embryo*	1 DPF	2 DPH	2 DPH	22 DPH
2	1	Embryo*	5 DPF	2 DPH	2 DPH	22 DPH
3	1	Embryo*	18 DPF	2 DPH	2 DPH	22 DPH
4	2	Larvae	2 DPH	9 DPH	3 DPH	26 DPH
5	2	Larvae	11 DPH	18 DPH	12 DPH	27 DPH
6	2	Larvae	26 DPH	33 DPH	27 DPH	58 DPH
7	2	Larvae	46 DPH	53 DPH	47 DPH	64 DPH
8	3	Young juvenile	60 DPH	89 DPH	61 DPH	89 DPH

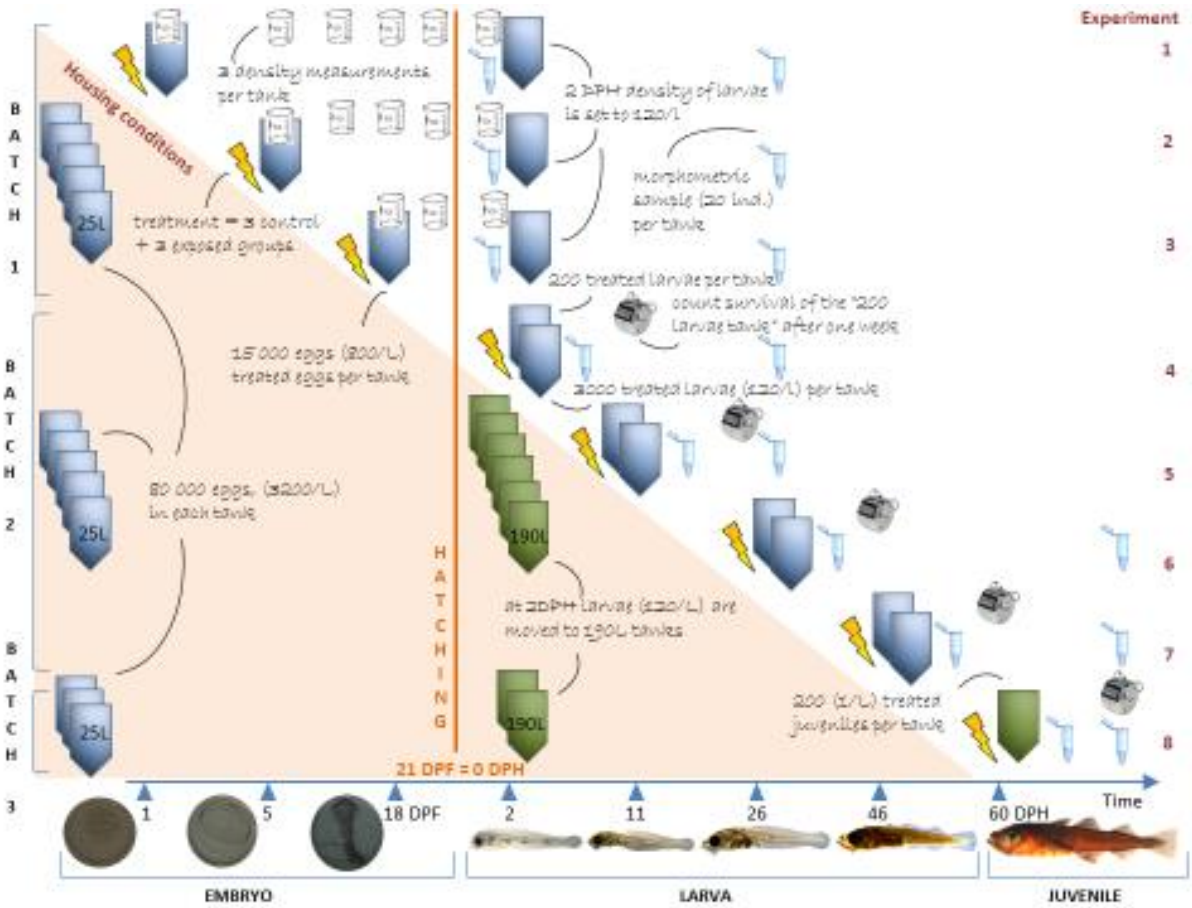


Figure 4.1: Schematic overview of the experimental procedure. On the left different batches and housing conditions are demonstrated. When time proceeds on the X-axis the eight different experiments are performed on the specific developmental stage. Legend: = 25L tank; = 190L tank; = treatment (3 control and 3 exposed groups in total); = density measurements (3/tank); = morphometric sample (20 ind./tank); = count survival.

4.2.2. Housing and rearing

All organisms were cultured according to protocols applied at the Centre for Marine Aquaculture Research (Hansen and Puvanendran, 2010; Hansen et al., 2015). The embryos (3200 L^{-1}) were housed in 25 L black cylindroconical tanks. Larvae (120 L^{-1}) were transferred at two DPH to green cylindrical tanks of 190 L supplied with aeration (Hansen et al., 2015). Seawater was provided to all tanks with a flow through system connected to the nearby fjörd (salinity, 32 ‰; pH, 8.1; oxygen, 9 mg L^{-1}; NH_3, <0.004 mg L^{-1}). Water temperature ranged between 4.0 - 4.5 °C for the embryos and it was gradually raised to 10 °C for larvae from 5 to 10 DPH. Two ml of algae (*Nannochloropsis*, Reed Mariculture, Campbell, CA, USA) per day were provided from 2 DPH until 12 DPH. From 2 DPH until 29 DPH and from 25 DPH until 55 DPH, rotifers and *Artemia franciscana* nauplii were delivered as live food, respectively. Prey densities (5-10 rotifers mL^{-1}; 1-10 *Artemia* mL^{-1}) were increased during rearing. At 38 DPH, larvae to be exposed as juveniles received 15 g dry feed (AlgaNorse Extra, Trofi AS, Tromsø, Norway) each day. This amount was increased to 60 g at 57 DPH, while *Artemia* prey densities were decreased gradually before they were discontinued (Hansen et al., 2015). Dead embryos and larvae were removed daily and 2-3 times every week, respectively.

4.2.3. Exposure to electric pulses

Before each exposure the number of embryos or larvae in the incubator tank was estimated by counting the organisms in a 50ml vial. In this way, the appropriate number of embryos (15,000) or larvae (3,200) was transferred from the incubator tank to a plastic 33 x 24.5 x 21 cm exposure chamber that contained 12 L of seawater (Figure 4.2). Within the chamber, two plate-shaped stainless steel electrodes (32 x 23 x 0.4 cm), conformed to the cross-sectional area of the chamber, were fixed in parallel at 24.5 cm apart and connected to the output of an adjustable laboratory pulse generator (LPG, EPLG bvba, Belgium) (Stewart 1972; Bohl et al., 2010; Henry and Grizzle 2004). The LPG generator was set to produce a unipolar square-wave pulsed direct current. Electrical output settings generated were 5 Hz frequency and 500 µs pulse duration resulting in a 0.25% duty cycle, similar to the pulse used to catch brown shrimp at sea (Verschueren and Polet 2009; Verschueren and Vanelslander, 2013). To create a homogeneous electric field of approximately 150 V/m an intensity of 36 V_{peak} was applied. The embryos, larvae and young juveniles were exposed for 5 s while being orientated in random directions.

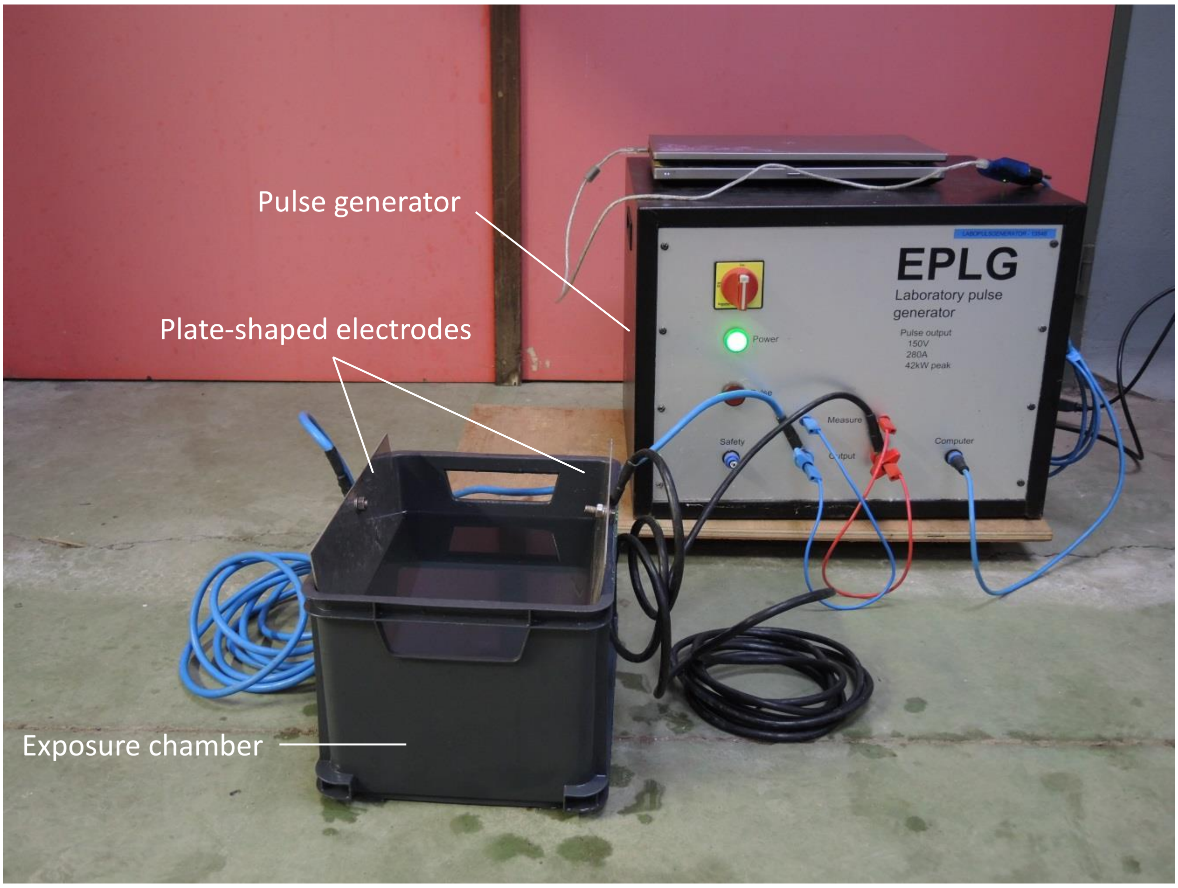


Figure 4.2: Two plate shaped electrodes connected to the pulse generator created a homogeneous electric field to which specimens were exposed.

4.2.4. Experimental set up

Exposure of embryos

Approximately 15,000 embryos were exposed once at each stage 1, 5 or 18 days post fertilisation (DPF) (experiment 1, 2 and 3, respectively). Following exposure, each group of 15,000 embryos was transferred to a new cylindroconical 25 L incubator (600 L^{-1}). In total 3 exposure and 3 control tanks were occupied at each developmental stage. The embryo mortality rate was estimated by counting the number of viable embryos in triplicate 50 ml vials taken from each aerated tank every 5 days until 23 DPF (2 DPH) at which time all embryos hatched into larvae.

During the hatching process at 21 DPF (0 DPH), the hatching/developmental rate was examined by counting the proportion of hatched larvae and the number of viable organisms in three 50 mL vials per tank. Dead embryos were removed daily until 2 DPH. At 2 DPH the number of larvae per tank was estimated and standardized at 3,000 larvae in 25 L^{-1} (120 L^{-1}). After hatching, 20 larvae per tank were sampled on a weekly basis until 53 DPH for a morphometric analysis described below.

Exposure of larvae

Electric pulses were given to 3,200 larvae at each stage 2, 11, 26 or 46 DPH (experiment 4, 5, 6 and 7, respectively). Following exposure, the animals were maintained in two subgroups of exactly 200 (8 L^{-1}) and approximately 3,000 larvae (120 L^{-1}) in two 25 L cylindroconical tanks. In total 12 tanks (6 exposure and 6 control tanks) were occupied at each developmental stage. At one week post exposure, the surviving larvae in the former subgroup of 200 larvae were counted and sacrificed. From the latter subgroup of 3000 larvae, 20 larvae were sampled every week until 58 DPH for morphometric analysis.

Exposure of juveniles

At 60 DPH (experiment 8), 200 juveniles were counted, exposed and maintained in a 190 L tank (1 L^{-1}). In total 3 control and 3 exposure tanks were occupied. On a weekly basis, 20 juveniles per tank were sampled for morphometric analysis and processed as described below. At 89 DPH, the surviving animals were counted and sacrificed.

4.2.5. Morphometric analysis

All sampled specimens were sacrificed with an overdose of 0.7 g L^{-1} MS 222 (Sigma-Aldrich, Oslo, Norway). The animals were then fixed in a 3% buffered glutaraldehyde solution and stored in 10 mL vials (Glauert, 1987) for morphometric analysis. For each developmental stage, the samples of two time points were processed for morphometric analysis as described below. The first time point was 1 DPH for the embryonic stages (experiment 1-3) and one day post exposure for the larval and juvenile stages at 3, 12, 27, 47 and 61 DPH (experiment 4-8). For all exposed embryonic stages, the second time point was 22 DPH. Larval stages exposed on 2, 11, 26 and 47 DPH were subjected to a morphological analysis at 26, 27, 58 and 64 DPH, respectively (Table 4.1). The second sample point for juveniles was 89 DPH. Specimens were photographed with AnalySIS GetIT software with an Olympus Altra 20 digital camera mounted on an Olympus SZX9 microscope equipped with an x 0.5 planar lens (www.olympus.com). Larvae were placed horizontally in a 100 µL seawater droplet on glass slides, with their left and right palatoquadrate cartilages vertically aligned (Nikolakakis et al., 2014). The straight notochord length, from the rostral tip of the upper jaw to the caudal tip of the notochord; the total notochord length, measured in segments from the tip of the upper jaw along the notochord to its caudal end; eye diameter, vertical eye length; head height, through the middle of the eye perpendicular onto the notochord; and muscle height, vertical length of the notochord muscle near the posterior tip of the gut or anus, were measured using ImageJ v1.46 (Figure 4.3). The ratio of straight notochord length on total notochord length was calculated as a measurement of the curvature of larvae/juveniles. Additionally, for the larvae sampled until 3 DPH, the yolk surface was measured.

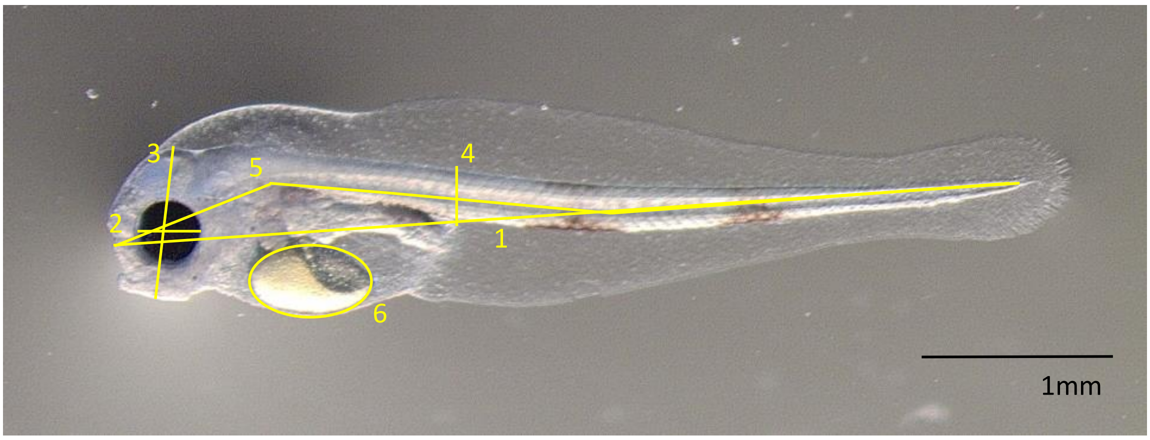


Figure 4.3: Overview of the measurements taken on 1 DPH larva; 1) Straight notochord length; 2) Eye diameter; 3) Head height; 4) Muscle height; 5) Total notochord length and 6) Yolk surface.

4.2.6. Statistics

According to the Shapiro-Wilk test, the embryo mortality rate was normally distributed. Therefore, the effect of the exposure on the embryo mortality rate was analysed by a mixed model. Replication was set as random effect and exposure, time and their interaction as categorical fixed effects. The analyses were done separately for each different exposure timing, i.e., at 1, 5 and 18 DPF. The effect of the exposure on the hatching/developmental rate at 0 DPH, and the mortality rate of larval and juvenile stages was analysed by a generalized mixed model with binomial error term (Stroup, 2012). Replication was set as random effect and developmental stage, exposure and their interaction as categorical fixed effects. The different length measurements were analysed by a mixed model with replicate as random effect and sample time, developmental stage, treatment and interaction between developmental stage and treatment as fixed effects.

4.3. Results

No significant differences in embryo mortality rate were found when exposure took place at 1 ($F_{1,32}$=0.04; P=0.837), 5 ($F_{1,26}$=0.84; P=0.369) or 18 ($F_{1,14}$=0.08; P=0.776) DPF (Figure 4.4). All groups started hatching on 20 DPF. Fifty percent hatching (0 DPH) occurred on 21 DPF. In the beginning of the hatching process, at 0 DPH, no significant differences in hatching/developmental rates were encountered between control and exposed groups for embryos exposed at 1 or 5 DPF, but a significant difference occurred in the embryos exposed at 18 DPF (OR=1.43, P=0.024), with a lower initial hatching/developmental rate in the exposed group (0.27, 95% CI:[0.23;0.32]) compared to the control group (0.35, 95% CI:[0.30;0.40]) (Figure 4.5). However, survival of larvae at 2DPH did not differ significantly from untreated controls ($F_{1,14}$=0.08; P=0.776).

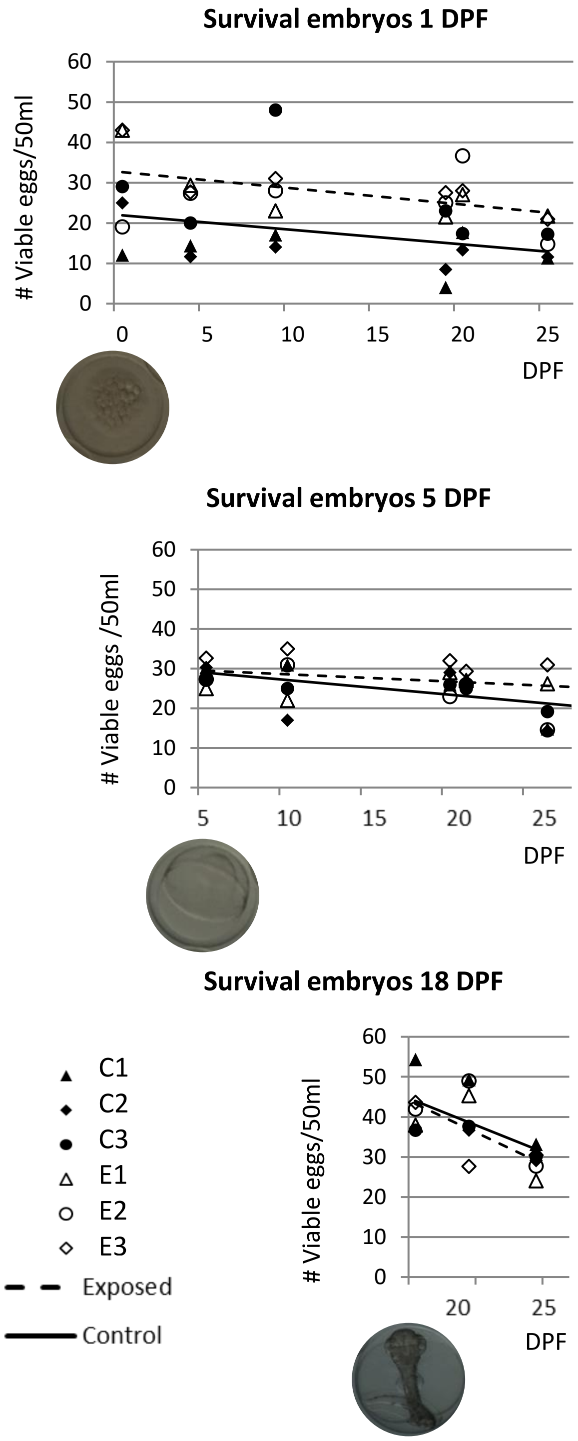


Figure 4.4: Survivorship of cod embryos exposed on 1, 5 and 18 DPF.

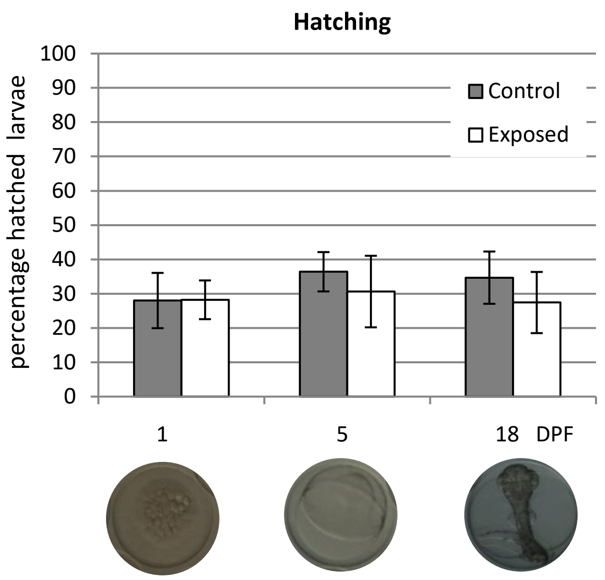


Figure 4.5: Hatching/developmental rate of cod embryos at 21DPF (0 DPH) that were exposed to electric current at 1, 5 and 18 DPF.

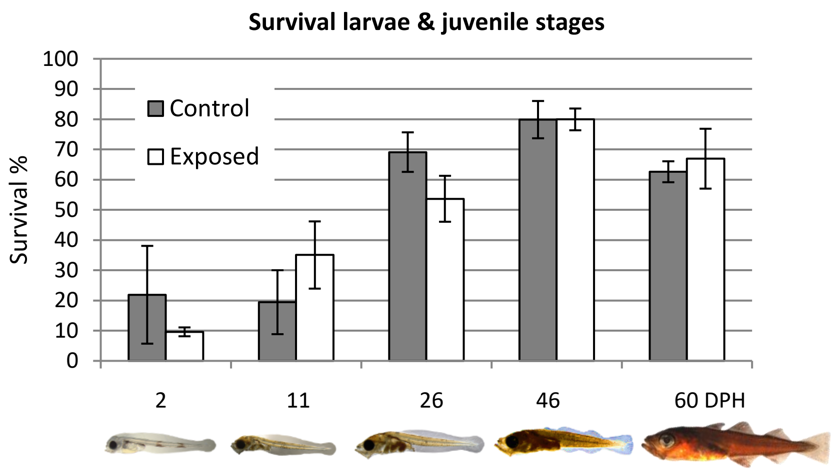


Figure 4.6: Short-term survival rates of cod larvae exposed at 2, 11, 26, 46 DPH and as juveniles (60 DPH) to an electric field and unexposed controls at the same time points.

In the trials investigating larval survival (Figure 4.6), survival differed significantly between the exposed and control groups when exposed at 2, 11 and 26 DPH, but not for later exposures at 46 and 60 DPH. At 2 DPH, a lower survival percentage (P=0.033) was observed in the exposed group (0.10, 95% CI:[0.08;0.11]) as compared to the control group (0.22, 95% CI:[0.10;0.40]). For 26 DPH exposure, this difference was even larger (P=0.001), with survival percentage in the exposed group equal to 0.53 (95%

CI:[0.46;0.61]) and in the control group 0.69 (95% CI:[0.63;0.75]). At 11 DPH, a higher survival percentage (P=0.048) was observed in the exposed group (0.35, 95% CI:[0.25;0.46]) as compared to the control group (0.19, 95% CI:[0.11;0.31]).

No significant differences between control and exposed groups at any of the morphometric measurements (all P>0.527), nor for the incurvation ratio (P=0.166) were observed at sample points one or two (Table 4.2).

4.4. Discussion

Cod embryos were exposed during early-cleavage, epiboly and near hatching on 1, 5 and 18 DPF, respectively. No differences in embryo mortality rate between exposed cod embryos and controls were found at any of the three egg stages. Reported effects on early life stages, limited primarily to salmonids, are often contradictory (Snyder, 2003). Nevertheless, a sufficient number of studies indicate that electrofishing in freshwater over spawning grounds may harm embryos on, or in, the substrate. Survival was affected, particularly when exposure happened between pre-cleavage and eyed-egg stages (Godfrey, 1957; Lamarque, 1990). This early stage of development was also most vulnerable when exposed to mechanical shocks (Kolz and Reynolds, 1990; Dwyer et al., 1993). According to Rollefsen (1930) younger cod embryos in early-cleavage, are more susceptible towards external influences than embryos at later stages as they are only covered by a thin layer of protoplasm. After the completion of epiboly during gastrulation, the yolk is covered by a thin layer of embryonal tissue resulting in increased resistance to external influences. Mortality before epiboly is completed may therefore be caused by rupture of the vitelline membrane or the protoplasm layer of the yolk (Hayes 1949; Godfrey 1957). Breakdown of the cell membrane may also occur when pores created during electroporation fail to reseal (Chen et al., 2006). Epiboly has hence been identified as the most sensitive stage during development to these stressors in different species (Muth and Rupert, 1997; Roach, 1999; Henry and Grizzle, 2004).

Table 4.2: Morphometric analysis on triplicate groups of 20 cod larvae and juveniles each that were exposed to electric current. C= control, E= exposed

Exposed	Sample	Treatment	Straight Length (mm)	StdDev (mm)	Total Length (mm)	StdDev (mm)	Ratio straight length/ Total length	Eye Diameter (mm)	StdDev (mm)	Head Height (mm)	StdDev (mm)	Muscle Height (mm)	StdDev (mm)	Yolk surface (mm²)	StdDev (mm²)
1 DPF	**1DPH**	**C**	4.732	0.167	4.788	0.162	0.988	0.327	0.023	0.760	0.036	0.255	0.017	0.147	0.050
		E	4.733	0.161	4.790	0.167	0.988	0.317	0.023	0.754	0.039	0.247	0.018	0.134	0.048
	22DPH	**C**	7.518	0.560	7.721	0.589	0.974	0.603	0.079	1.524	0.148	0.526	0.089		
		E	6.928	0.581	7.111	0.602	0.974	0.552	0.066	1.396	0.183	0.457	0.081		
5DPF	**1DPH**	**C**	4.662	0.263	4.779	0.144	0.975	0.329	0.022	0.743	0.040	0.244	0.018	0.128	0.046
		E	4.691	0.181	4.769	0.156	0.984	0.333	0.029	0.742	0.072	0.246	0.018	0.138	0.060
	22DPH	**C**	6.142	0.632	6.257	0.631	0.982	0.482	0.073	1.196	0.186	0.354	0.090		
		E	6.452	0.702	6.596	0.722	0.978	0.506	0.074	1.274	0.193	0.399	0.096		
18DPF	**1DPH**	**C**	4.635	0.203	4.725	0.199	0.981	0.331	0.027	0.756	0.061	0.249	0.016	0.139	0.051
		E	4.615	0.237	4.725	0.151	0.977	0.331	0.023	0.752	0.069	0.251	0.017	0.129	0.051
	22DPH	**C**	7.326	0.564	7.517	0.571	0.975	0.596	0.070	1.483	0.174	0.525	0.089		
		E	7.150	0.679	7.327	0.711	0.976	0.574	0.076	1.422	0.188	0.494	0.103		
2DPH	**3DPH**	**C**	4.810	0.148	4.891	0.133	0.983	0.335	0.020	0.768	0.033	0.242	0.020	0.041	0.019
		E	4.732	0.369	4.863	0.233	0.973	0.322	0.028	0.740	0.071	0.242	0.024	0.043	0.029
	26DPH	**C**	7.403	0.768	7.550	0.779	0.981	0.551	0.067	1.414	0.166	0.492	0.093		
		E	7.512	0.552	7.689	0.553	0.977	0.567	0.059	1.474	0.122	0.511	0.066		
11DPH	**12DPH**	**C**	5.906	0.359	6.018	0.368	0.981	0.442	0.036	1.038	0.082	0.335	0.045		
		E	6.007	0.356	6.106	0.352	0.984	0.447	0.033	1.049	0.064	0.340	0.038		
	27DPH	**C**	7.287	0.576	7.438	0.588	0.980	0.564	0.060	1.411	0.157	0.476	0.077		
		E	7.117	0.626	7.295	0.658	0.976	0.558	0.070	1.384	0.158	0.439	0.081		
26DPH	**27DPH**	**C**	7.953	0.615	8.144	0.625	0.977	0.671	0.061	1.623	0.217	0.562	0.095		
		E	7.839	0.726	8.018	0.750	0.978	0.645	0.072	1.605	0.180	0.552	0.103		
	58DPH	**C**	11.190	1.414	11.433	1.454	0.979	1.174	0.167	2.641	0.392	1.319	0.266		
		E	11.893	1.899	12.137	1.901	0.980	1.219	0.199	2.917	0.558	1.488	0.371		
46DPH	**47DPH**	**C**	10.303	1.534	10.570	1.543	0.975	1.027	0.163	2.461	0.396	1.162	0.290		
		E	9.974	1.385	10.257	1.362	0.972	1.016	0.140	2.306	0.378	1.105	0.278		
	64DPH	**C**	18.070	2.697	18.239	2.739	0.991	1.638	0.193	4.078	0.499	2.432	0.430		
		E	16.749	2.693	16.861	2.735	0.993	1.534	0.204	3.837	0.547	2.270	0.444		
60DPH	**61DPH**	**C**	17.135	2.601	17.276	2.621	0.992	1.623	0.226	3.698	0.533	2.295	0.427		
		E	17.885	2.820	18.088	2.834	0.989	1.668	0.213	3.737	0.547	2.396	0.492		
	89DPH	**C**	43.376	6.079	43.874	6.171	0.989	3.304	0.457	8.823	1.537	5.777	1.061		
		E	42.457	6.020	42.960	6.071	0.988	3.257	0.430	8.903	1.471	5.713	0.924		

In contrast, several hypotheses as to why electric pulses did not elicit a negative impact on these vulnerable life stages may be advanced. Cod eggs are relatively small, 1.16 – 1.89 mm (Andersen et al., 1994; Auditore et al., 1994). As transmembrane potential increases with cell radius (Gaylor, 1988), several studies confirmed that electroshock-induced mortality increases with egg size (Henry and Grizzle, 2004; Bohl et al., 2010). Survival is known to decrease when voltage levels increase. A voltage gradient of 8-16 V/cm DC was needed to cause significant mortality in fresh water species such as largemouth bass (*Micropterus salmoides*) and bluegill (*Lepomis macrochirus*) with a comparable egg size of approximately 1.7 and 1.1 mm respectively (Henry & Grizzle, 2004). In the present trials, a lower intensity of 1.5 V/cm was applied in seawater to the cod eggs to simulate the shrimp pulse. Also electric current type may be critical to embryo survival. Pulsed direct current as used in our experiments has resulted in higher survival than direct currents (DC) (Dwyer and Erdahl, 1995; Keefe et al., 2000; Henry and Grizzle, 2004).

Electric fields may induce premature hatching, as observed in bluegill and medaka (*Oryzias latipes*), resulting in an *in situ* increased risk of predation and consequently higher mortality (Yamagami, 1988; Henry & Grizzle, 2004). In the current study, no higher hatching/developmental rate at 0 DPH was observed in exposed groups. In contrast, a lower initial hatching/developmental rate was noted for eggs exposed at 18 DPF. The reason for this finding is not clear, but might be attributed to chemical reactions with seawater induced by the electrodes. Indeed, electrolysis of the anode might release metal-ions in the environment and a secondary production of oxidants such as chlorine and bromine may occur (Stewart, 1972; Yalçin et al., 1997). Oxidants including ozone are known to delay or reduce the hatchability of cod eggs (Grotmol et al., 2003). They may modify the protein polymer compounds in the eggshell rendering it more resistant to hatching enzymes responsible for the weakening of this membrane. The secretion of these enzymes by the hatching gland may also be inhibited. In addition, low concentrations of possibly produced metal-ions and oxidants are well known to be toxic and reduce the survival of aquatic organisms (Stewart et al., 1979; Abarnou and Miossec, 1992; Arimoto et al., 1996). However, in the present study, electrolysis was probably minor because exposures of only

5 seconds were used and no differences in survival rate were observed at 2 DPH. Additionally, at sea this phenomenon will be limited by the continuous abrasion of the electrode surface during towing at speeds of 3 knots (Stewart, 1972). Nevertheless, different chemical reactions might still be possible in the electrically trawled sediment, especially in substrates rich in organic matter and metals (Alvarez-Iglesias and Rubio, 2009; Soetaert et al. 2015). Another explanation for the delayed hatching/developmental rate could be that electric pulses might interfere with the frequency of sporadic muscular contractions that finally cause the chorion to tear (Hall et al., 2004).

The vulnerability of early life stages seems to decrease as their development proceeds. However, for some fresh water fish species, the above mentioned sensitive embryonic stages appear to be less susceptible to electrical stimulation than later post-hatching stages (Henry et al. 2003, Henry and Grizzle, 2003, 2006; Muth and Ruppert, 1997). In our experiment, a significantly lower survival rate was noted following exposure of larvae at 2 and 26 DPH in comparison with their corresponding control group. In this latter stage, many organ systems are developing (Yin and Blaxter 1986; Pedersen and Falk-Petersen, 1992; Brown et al., 2003). Additionally, this stage is more sensitive to external stress because of its transition from cutaneous to gill respiration (Herbing et al., 1996). Besides, larvae are feeding on *Artemia* and need to chase their prey actively with their yolk completely depleted. In general, this developmental stage is known to be a bottleneck in cod larviculture conditions with failure to initiate and maintain sufficient feeding being the major source leading to mass mortality (Puvanendran and Brown, 1999, 2002; Brown et al., 2003). In contrast, cod at later developmental stages, larvae in metamorphosis and juveniles display higher survival rates and appear to be more robust (Pedersen and Falk-Petersen, 1992; Opstad et al., 2006; Meier et al., 2010). Indeed, in our studies, no differences in survival were found during metamorphosis or in the juvenile stage of cod exposed at 46 or 60 DPH, respectively.

In the present study, a homogeneous electric field of approximately 150V/m for 5 s was applied as a worst-case scenario to randomly orientated animals. Orientation and position in the electric field are important as the highest head to tail voltage will be experienced when animals are orientated perpendicular to the electrodes. At sea, a heterogeneous electric field distribution is created for less than 2 seconds on the assumption that individuals are at rest and only exposed when 150 cm long electrodes are passing by at a speed of 3 knots. A heterogeneous field implies that field strengths are higher in close proximity (up to 150 V/m at 5 cm) and lower when the distance to the electrode increases (approximately 30 V/m at a moderate distance of 30 cm) (Verschueren and Polet 2009; Verschueren et al., 2013). The latter is presumed to be the case for the majority of organisms. Indeed, the potential for cod embryos and larvae to be exposed to an electric field of 150 V/m during electrofishing will be low as they are pelagic and buoyant (Fahay, 1983; Markle and Frost, 1985) while the electric field will be limited to the net opening of the trawl. However, turbulent forces such as mixing forces from wind may distribute pelagic life stages in a downward direction increasing their chances for contact with the electric field (Sundby, 1983; Conway et al., 1997). Therefore, young buoyant life stages of cod may have higher chances of contact with the electric field in their shallow coastal spawning areas (Munk et al., 2002) where shrimp trawling often occurs. Cod larvae move deeper as they become older (Yin and Blaxter, 1987; Heesen and Rijnsdorp, 1989) and descend from the water column to bottom habitats at sizes of 2.5-6 cm, when a complete transformation to the juvenile stage occurs (Fahay 1983; Lough et al., 1989). Thus, it is more likely that these developmental stages will be in contact with the electrofishing equipment. However, no significant differences in mortality compared to controls were noted for cod exposed during or after metamorphosis to the young juvenile stage at 46 and 60 DPH, respectively. Nevertheless, the impact on older juveniles larger than 2.4 cm was not examined in our trials.

No significant differences in morphometric parameters between exposed and control organisms were found, indicating that growth rate was not affected by electric field exposure. Furthermore, morphometric changes, such as jaw deformities which are known to prevent feeding (Tilseth et al., 1984; Meier et al., 2010), abnormal yolk resorption, increased incurvation or deformations, such as lordosis and scoliosis, did not differ between exposed and control groups.

Eggs were obtained from three batches to ensure that all embryos, larvae and juveniles to be compared were the same age in each experiment to reduce variability in hatching percentage and egg and larval quality between replicates. Indeed, inconsistency in growth rates and survival among tanks is one of the major problems encountered with intensive cod larval rearing (Thorsen et al., 2003; Hamre, 2006; Monk et al., 2006). These phenomena introduce complications in interpreting results from studies of fish larvae as was the case in the present study for the larvae exposed at 2 and 11 DPH. We are hesitant to draw any conclusions from these data especially since the differences in survival were borderline significant. This is in contrast with the findings for the 26 DPH larvae, where the difference between exposed and control groups was much greater.

The present research is innovative in that it is the first to examine the impact of electric pulses on a marine fish species during its embryonic, larval and young juvenile stages employing cod as a model species. However, follow-up studies are necessary to fully grasp the potential impact of pulse trawls on these young life stages and reproductive success of adults (Cho et al., 2002). Indeed, studies on the impact of electric pulses on the reproduction of adult brood stock and on fertility success of exposed gametes are lacking. Exposure of ripe female fish to electric fields may cause significant damage or premature expulsion of gametes and reduced viability of subsequently fertilized eggs (Muth and Ruppert 1997; Roach 1999). Therefore, a greater proportion of abnormal cod larvae hatching from eggs of stressed females may be produced (Morgan et al., 1999). Although multiple exposures with intervals of 1-5 min did not appear to cause major harm to zebrafish embryos (Nutile et al., 2012), research on the effects of electrofishing on young marine organisms is limited to single exposure events. Information on the impact of multiple exposures is important, as certain fishing grounds including spawning areas may be fished intensely during particular seasonal periods (Piet and Hintzen, 2012; van Denderen, 2015). In addition, other marine species should be included such as flatfish. During larval development, these fish species demonstrate very complex morphological changes during metamorphosis, such as migration of the eye (Palazzi et al., 2006; Piccinetti et al., 2012) and could therefore be more vulnerable to electric pulses. Other species, such as herring, also need to be investigated since demersal eggs are produced (Yin and Blaxter, 1987) which could be exposed when electrodes are towed over the sea bed.

Acknowledgments:

This research received funding and support from the Agency for Innovation by Science and Technology in Flanders (IWT), The European Fisheries fund (EFF), Aquaexcel and the staff at the Centre for Marine Aquaculture Research in Tromsø.

CHAPTER 5: Pulse trawling: The impact of pulsed direct current on early life stages of sole (*Solea solea*)

This chapter is based on

Desender, M., Dumolein, L., Duchateau, L., Adriaens, D., Delbare D., Polet, H., Chiers, K., and Decostere, A. 2018. Electrotrawling: The impact of pulsed direct current on early life stages of sole (*Solea solea*). (In Press: North American Journal of Fisheries Management)

Abstract

Despite electric pulse fishing being regarded as a promising environmentally friendly fishing method, very little is known about possible negative effects on early life stages of bottom dwelling species such as sole (*Solea solea*). Electrotrawling for brown shrimp is increasingly used in shallow coastal zones and estuaries of the southern North Sea. As these fishing grounds are often important nurseries and spawning areas for various marine species, electrotrawling could therefore harm inhabiting fishes during their early life stages. Hence, this research aims to investigate the effect of electric pulses used to catch brown shrimp on the survival and development of sole embryos and larvae. Exposure of sole embryos at 2 days post fertilisation and larvae at 11 days post hatching to pulsed direct current used to catch brown shrimp did not result in a lower survival eight days post exposure. Additionally, no differences in yolk sac resorption and morphometric length measurements of the notochord, muscle, eye and head, were observed in the developing larvae.

Keywords: Electrotrawling, pulse trawling, pulsed direct current, life stages, sole, survival, development, morphometrics

5.1. Introduction

Electrotrawls, also named pulse trawls, are increasingly used to catch marine benthic species such as brown shrimp (*Crangon crangon*) and flatfish including sole (*Solea solea*) and plaice (*Pleuronectes platessa*) (Verschueren and Polet, 2009; Van Marlen et al., 2014; Soetaert et al., 2015). In these devices, the mechanical stimulation by tickler chains, chain matrices and bobbins equipping traditional beam trawls, is replaced by electrodes. Although fishing by means of electric currents in the sea is prohibited since 1988 in Europe (EU, 1998), derogations manifested in 2009 and 2013 resulted in approximately 91 pulse trawls currently operating in the southern North Sea (EU, 2009; 2013).

In the electrotrawl used to catch brown shrimp, a field strength of 30 V m^{-1} in the middle between thread shaped electrodes (Ø 1.2 cm; 150 cm) is generated by using a low-frequency 5 Hz pulsed direct current (PDC) of 0.5 ms (Verschueren and Polet, 2009; Verschueren et al., 2012). This electrical field selectively induces a startle response by contraction of the abductor muscles (tail-flip) in the shrimp (Polet et al., 2005a). Consequently, the majority of other benthic organisms remain on the seafloor and can escape underneath a hovering trawl that collects the jumping shrimp.

In the original electrotrawl targeting brown shrimp, the hovercran, all bobbins are replaced by 12 light-weight electrodes (Verschueren and Polet, 2009; Verschueren et al., 2012). However, at sea, vessels differ in fishing grounds and consequently rigging, gear configuration and number of bobbins used, resulting in different outcomes considering selectivity and bottom contact (Verschueren and Vanelslander, 2013; Verschueren et al., 2014). Experiments of the pulse implemented with a reduced amount of bobbins, 11 instead of 36 as used in traditional gear, further diminished bycaught animals of all lengths with 76% in comparison with the conventional beam trawl. Also, seabed contact was reduced by 60% resulting in 23% less drag resistance (Verschueren et al., 2014).

Despite electric pulse fishing being regarded as an environmentally friendly fishing method, concerns are raised amongst various stakeholders (Kraan et al., 2015). An electric pulse field in the net opening of a traditional shrimp trawl may significantly increase its catching efficiency for the target species. During monitoring experiments performed from June till October (Verschueren et al., 2014; 2016), between 9% and 30% more market sized shrimps were caught. This especially is due to the fact that the pulse trawl allows to efficiently catch shrimps during daytime and clear water which is in contrast with traditional gear which is most efficient in catching shrimps at night or in turbid water. This increased catching efficiency of the pulse trawl may benefit the fishery in two ways. On the one hand the trawl may be designed to be more selective, i.e. reduce the unwanted by-catch while also loosing part of the target species. As such the amount of landed shrimp can remain equal to the traditional trawl while significantly reducing discards. On the other hand, fishermen may choose to use the increased catching efficiency for the target species to land more shrimps while keeping the discards at the same level of the traditional trawl. This choice has important consequences for the management of the fishery. Choosing the first option does not change the pressure on the shrimp stock. As such the same fleet, employment and fishing effort may be maintained while reducing the environmental impact of the fishery. Choosing the second option increases the fishing capacity of the fleet which is in contradiction with the management target of the EU Common Fisheries Policy to not increase the fishing capacity of EU fishing fleets. A consequence may be that the size or effort of the shrimp trawler fleet should be reduced to keep the pressure on the target stock at the same level. Currently the shrimp fishery is largely unregulated as stocks are hitherto considered sustainable. However, nowadays there are strong indications that high fishing pressure likely has led to growth overfishing of the brown shrimp population in the North Sea (ICES, 2015; Tulp et al., 2016). An example of pulse trawling in China has demonstrated the potential danger of increased fishing capacity by technological creep. Unregulated exploitation accompanied with more efficient electrotrawling devices led to a drastic decline in shrimp biomass in the East Chinese Sea in the 1990's (Yu et al., 2007). Fortunately in the North Sea brown shrimp fisheries, steps are being taken towards a better brown shrimp stock management (ICES, 2013; ICES, 2015). In the frame of eco-labelling for brown shrimp, the fishing industry has proposed a landing per unit effort (lpue) based harvest control rule (ICES, 2014). Guided by this measure, this risk coming with an introduction of a shrimp pulse trawl should be low, although it may lead to a gradual reduction of the fleet. This reduction may be avoided if the pulse trawl is used to reduce discards rather than increasing the catches of the target species.

Concerns are also increasing regarding the impacts on targeting or neighbouring non-target organisms (Yu et al., 2007). Electric fields as adopted in the shrimp pulse seem to have only limited impact on cod (*Gadus morhua*), sole (*Solea solea*) and other adult marine organisms (Polet et al., 2005a; Desender et al., 2016; Soetaert et al., 2014; Soetaert et al., 2016). However, very little is known about possible negative effects of pulsed direct current during different early life stages of bottom dwelling species. Brown shrimp are often targeted in shallow coastal waters and estuaries such as the Wadden sea. This area is one of the most important recruitment areas of the Northeast Atlantic sole stocks (Rijnsdorp et al., 1992). Therefore, it is crucial to address the hypothesis that electrotrawling over such fishing grounds may harm vulnerable embryos, larvae and/or juveniles (Snyder, 2003). Hence, the present study aimed to investigate the impact of electric pulses used to catch brown shrimp on the development of sole embryos and larvae and using survival, yolk sac surface and morphometric length measurements as response variables.

5.2. Materials and methods

5.2.1. Experimental animals and housing

Naturally fertilized sole (*Solea solea*) embryos purchased from captive broodstock maintained at the Institute for Marine Resources & Ecosystem Studies (IMARES, Ijmuiden, the Netherlands) were incubated in cylindroconical 9.5 L containers at the Institute for Agricultural and Fisheries Research (ILVO, Ostend, Belgium). The tanks were provided with aeration and UV-treated sea water of 34‰ salinity in a half-enclosed recirculation system. The embryos and larvae were reared in these housing tanks until treatment at 2 day post fertilization (DPF) and 11 days post hatching (DPH). The water temperature was set at 15 °C. Hatching occurred at 4 DPF, which was referred to as 0 DPH in larval age. From 3 DPH onwards, larvae were fed every day with *Artemia franciscana* nauplii (4 ind. ml^{-1}).

All experiments were approved by the ILVO animal experimental ethical committee (EC/2012/171).

5.2.2. Exposure system

An adjustable laboratory pulse generator (LPG, EPLG bvba, Belgium) produced a unipolar half square-wave pulsed direct current (PDC). Generated electric output settings were 5 Hz frequency and 500 µs pulse duration, corresponding to the pulse used at sea to catch brown shrimp (Verschueren & Polet 2009; Verschueren et al., 2012). Two plate-shaped stainless steel electrodes (10 x 15 x 0.4 cm) were connected to the output of the LPG and parallel fixed at 18 cm apart matching the cross-section of a plastic exposure chamber (18 x 10 x 15 cm) (Henry & Grizzle 2004; Desender et al., 2017). An intensity of 25 V peak was applied to create a homogeneous electric field of approximately 150 V m^{-1}. Before the onset of each experiment, the electric characteristics were confirmed using a digital oscilloscope (Tektronix TDS 1001B).

5.2.3. Experimental set-up

The embryos and larvae were exposed once for 5 s to a 150 V m^{-1} electric field in the exposure chamber. The latter was refilled after each exposure with 2.4 L of seawater. Ten groups of 0.84 ± 0.23 g embryos were treated at 2 DPF. Another 10 control groups of 0.88 ± 0.20 g embryos were subjected to the same handling as the exposed groups except that the electric field was not switched on. Electric pulses were additionally given to 7 groups of 24 ± 2 larvae at 11 DPH. Seven control groups of 26 ± 5 larvae were also included. The larvae exposed at 11 DPH were not exposed earlier in the 2DPF experiment. Following exposure, each group of embryos or larvae was incubated in a 1 L container until eight days post exposure. The containers were provided with aeration, and water renewal every second day.

At eight days post exposure, that is 6 DPH for embryonic and 19 DPH for larval stages, the number of surviving specimens was counted. All individuals were sacrificed with an overdose of MS 222 (Sigma-Aldrich). Following, 20 animals out of each group were fixed in a 3% buffered glutaraldehyde solution and stored in a 10 mL vial (Glauert, 1987). Specimens were photographed with an Olympus Altra 20 digital camera mounted on an Olympus SZX9 microscope equipped with an x 0.5 planar lens and subjected to a morphological analysis via AnalySIS GetIT software (www.olympus.com). Larvae were placed on glass slides in 100 µL seawater., Their right and left palatoquadrate cartilages were aligned (Nikolakakis et al., 2014). The straight notochord length; the total notochord length; eye diameter; head height; and muscle height were measured using ImageJ v1.46 (Figure 5.1). The ratio straight notochord length to total notochord length was calculated as a measurement for the artificial incurvation of larvae. Samples were taken before the onset of notochord flection. Additionally, the yolk sac outline of larvae sampled at 6 DPH, was traced and the surface was computed.

The effect of the exposure on the survival of embryos was analysed with a likelihood-ratio test based on the Poisson model, with start weight as a fixed offset term and replication as random effect. The effect of the exposure on the mortality of the larval stage was analysed by a logistic regression analysis with replicate as random effect. The different length measurements were analysed by a linear mixed model with replicate as random effect and age, treatment and the interaction as fixed effects.

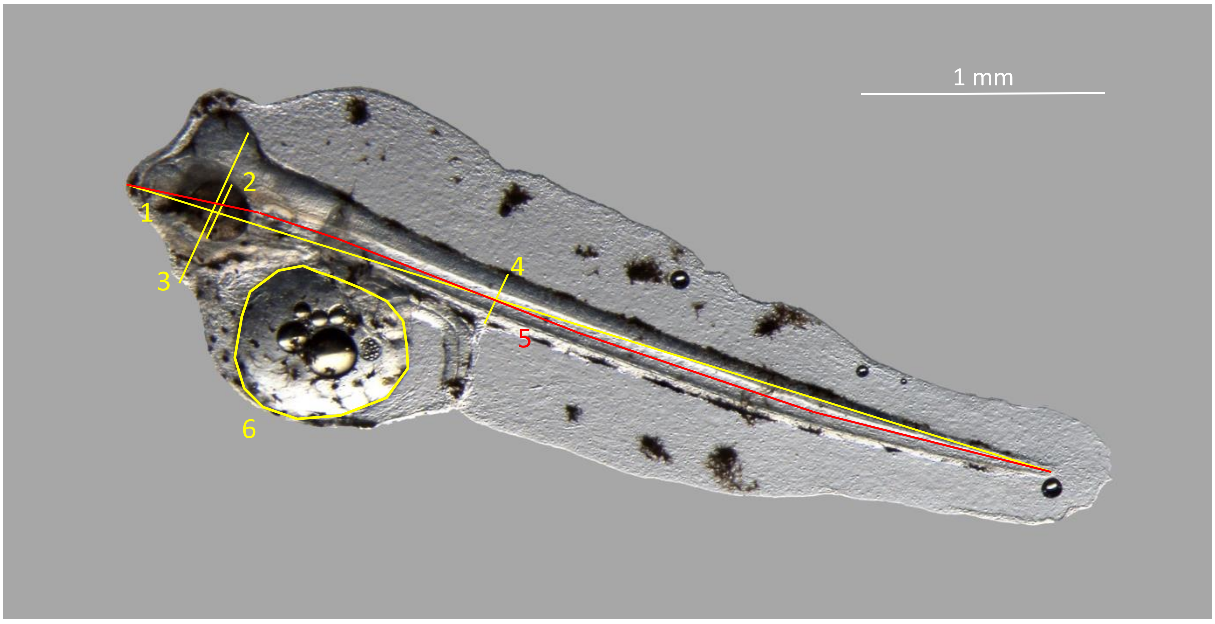


Figure 5.1: Overview of the measurements on a larva 6 DPH: 1) Straight notochord length; 2) Eye diameter; 3) Head height; 4) Musculature height; 5) Total notochord length; and 6) Yolk surface.

5.3. Results

At 3 DPH, a fungal infection was noted in the embryos. Therefore, a treatment with 2% hydrogen peroxide was installed (De Swaef et al., 2015).

A significant difference in survival was observed for the embryos treated at 2 DPF ($P<0.0001$) with higher survival observed in the exposed group (28.18 larvae/g embryos, 95% CI:[24.80;32.01]) as compared to the control group (17.74 larvae/g embryos, 95% CI:[15.15;20.76]) (Figure 5.2). Regarding the trials at 11 DPH involving larvae (Figure 5.3), survival did not differ significantly ($P=0.3262$) between the exposed (69.23%, 95% CI:[62.39;76.07]) and control groups (64.28%, 95% CI:[56.89;71.68]). No significant differences between any of the morphometric measurements (all $P > 0.4630$), nor for the incurvation ratio ($P = 0.4737$) or the yolk sac surface ($P= 0.3713$) were observed between control and exposed groups (Table 5.1).

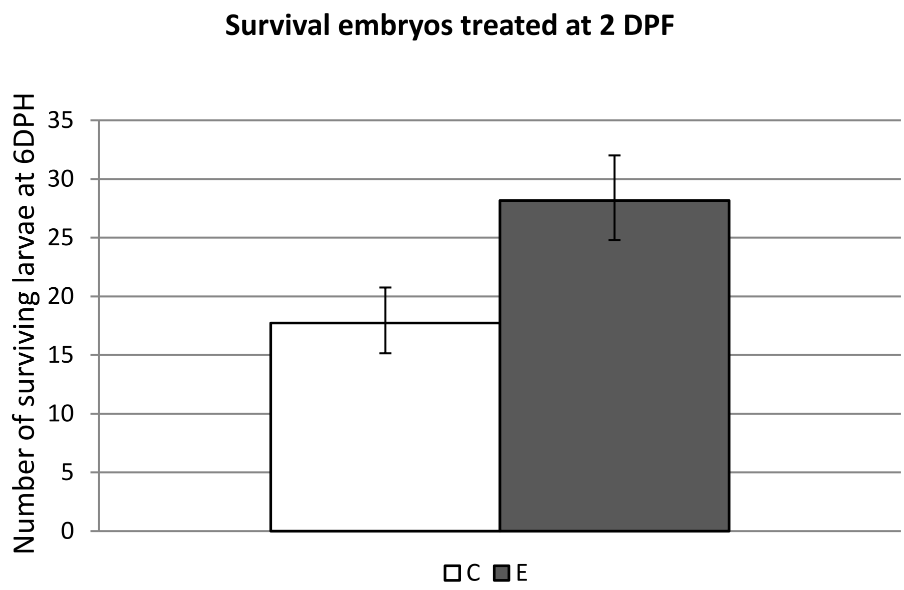


Figure 5.2: Counts of surviving larvae for the two treatment groups (C=control; E= exposed) at a start weight equal to one gram of treated embryos. DPF = Days Post Fertilisation.

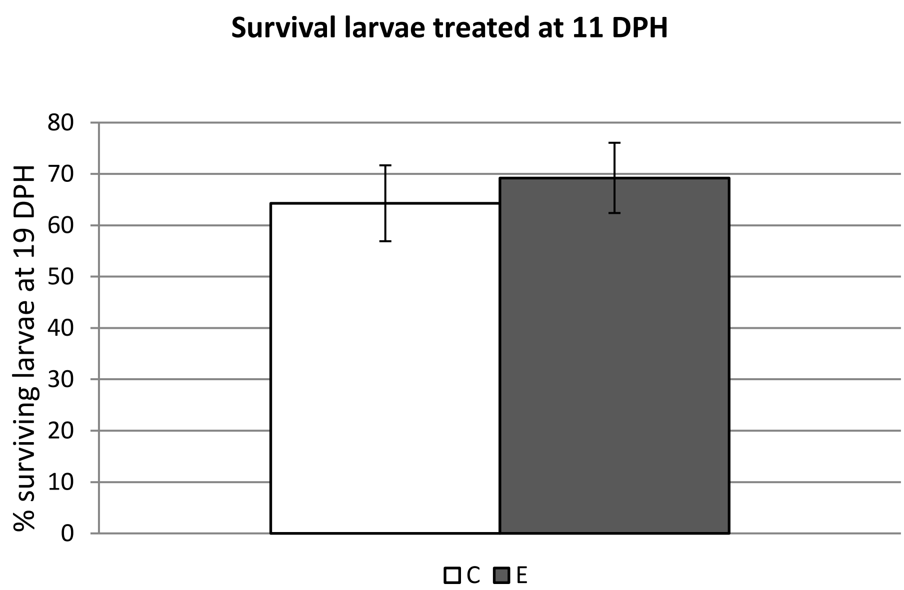


Figure 5.3: Counts of surviving larvae for the two treatment groups (C=control; E= exposed). DPH = Days Post Hatching.

5.4. Discussion

Adult sole exhibited a cramp or escape response during exposure to a pulse of more than 40 Hz and below 20 Hz, respectively. No gross or microscopic lesions, mortality nor deviant behaviour were observed (Desender et al., 2016; Soetaert et al., 2016). Data on the impact of electric pulses during the early life stages of marine flatfish, i.e. embryos or larvae, were fully lacking at the initiation of the present research. These data nevertheless are crucial as shrimp pulse trawling may occur in shallow coastal waters and estuaries, which are important nurseries and spawning areas for many marine organisms, including flatfish species such as sole.

Several studies conducted in freshwater indicated that early life stages may be highly sensitive to electric currents (Muth & Ruppert, 1997; Keefe et al., 2000; Henry et al., 2003), with the electric field, exposure duration, developmental stage and species playing a significant role (Snyder, 2003). However, one should consider that data resulting from the use of electric currents applied in freshwater with different electric settings cannot be extrapolated towards a marine environment due to differences in conductivity, justifying the current experiments.

In the present study, exposure of sole embryos at 2 DPF to PDC used to catch brown shrimp did not result in a lower survival 8 days post exposure. At 2 DPF the sole embryo is in epiboly. This stage at which a thin layer of embryonal tissue is growing over the yolk, is considered as the most sensitive stage during development in different species (Muth & Rupert 1997; Dwyer et al., 1993, Roach, 1999; Henry & Grizzle 2004; Bohl et al., 2010; Nutile et al., 2012). Also in cod embryos at epiboly, 5 DPF, survival was not negatively affected when exposed to the same pulse (Desender et al., 2017). In freshwater embryos, survival was particularly affected when exposure to mechanical shocks or electric pulses happened between pre-cleavage and eyed-egg stages (Godfrey 1957; Lamarque 1990; Kolz and Reynolds 1990). As mortality caused by electric pulses increases with egg size, a voltage gradient of 16 V cm^{-1} needed to be applied to cause a significant decrease in survival for freshwater species with a comparable egg size of approximately 1mm (Henry & Grizzle 2004; Bohl et al 2010). In the present trials, a lower intensity of 1.5 V cm^{-1} was applied in seawater, corresponding to the pulse used to catch brown shrimp at sea.

All groups, control and exposed, suffered from a fungal infection at 3DPH and consequently were treated with hydrogen peroxide. Egg disinfection is commonly employed as a disease management tool in aquaculture hatcheries (de Swaef et al., 2015). Disinfection with hydrogen peroxide was part of the protocol to pinpoint a reliable experimental set-up for Dover sole larvae with good survival rates (de Swaef et al., 2017). We do not expect that the disinfection nor the fungal infection in itself impacted our findings taking into account that both the exposed and control group were infected and treated. This being said, one might presume that the infection and treatment might have rendered the embryos more vulnerable to electrical pulses, hence creating a worst-case scenario.

Active *Artemia* feeding sole larvae exposed at 11DPH exhibited no increased mortality. This is in contrast with the former study where cod larvae at 26DPH, approximately the same developmental stage, showed a reduced survival (Desender et al., 2017). Additionally the yolk surface and morphometric length measurements of notochord, muscle, head and eye, indictors of growth or starvation, revealed no differences or deformations between treatment groups.

During exposure a worst-case scenario was performed by creating a homogeneous field of 150 V m^{-1}. At sea, a heterogeneous electric field distribution is created, which is limited to the net opening of the trawl. This implies that field strengths are higher in close proximity of the electrodes and lower when distance to the electrode increases. A field strength of 150 V m^{-1} in situ is only reached in a small part of the electric field, approximately 5 cm from the electrode. At a larger distance, which is presumed to be the case for the majority of organisms, a mere 30 V m^{-1} will be experienced at sea (Verschueren & Polet, 2009).

Table 5.1: Morphometric analysis data of 20 larvae sampled in triplicate for each treatment: straight length, total notochord length, eye diameter, head height and muscle height measurements (mm), yolk surface (until 6 DPH; mm^2) and the incurvation ratio straight length on total notochord length. C= control, E= exposed.

Exposed	Sample	Treatment	Straight Length (mm)	StdDev (mm)	Total Length (mm)	StdDev (mm)	Ratio straight length/ Total length	Eye Diameter (mm)	StdDev (mm)	Head Height (mm)	StdDev (mm)	Muscle Height (mm)	StdDev (mm)	Yolk surface (mm^2)	StdDev (mm^2)
2 DPF	**6 DPH**	**C**	3.746	0.443	3.788	0.393	0.986	0.264	0.025	0.662	0.075	0.259	0.020	0.174	0.075
		E	3.766	0.394	3.813	0.336	0.985	0.266	0.029	0.662	0.067	0.255	0.018	0.172	0.071
11 DPH	**19 DPH**	**C**	4.632	0.454	4.644	0.454	0.995	0.307	0.038	0.880	0.130	0.331	0.073		
		E	4.536	0.494	4.579	0.410	0.988	0.314	0.037	0.866	0.117	0.329	0.060		

This study suggests that sole embryos or larvae are not likely to exhibit increased short term mortality, deformations, reduced growth or differences in yolk sac resorption as a result of exposure to the electric field implemented in shrimp trawls. However, other sub-lethal effects, behavioral alterations or long-term effects were not addressed. As survival and growth alone may not reflect the complete physiological condition of a fish (Dhert et al., 1992; Logue et al., 2000; Lund, 2007), the precautionary approach is still warranted when making statements on the impact of electric pulses on early marine life stages.

Acknowledgments

The research leading to these results has received funding and support from the Agency for Innovation by Science and Technology in Flanders (IWT) and The European fisheries fund (EFF). Special thanks to Ewout Blom for supply of egg batches at the Institute for Marine Resources & Ecosystem Studies (IMARES, the Netherlands) and to David Vuylsteke for his help in maintaining eggs and larvae at the Institute for Agricultural and Fisheries Research (ILVO, Belgium).

CHAPTER 6: Pulse trawling: evaluating its impact on prey detection by small-spotted catshark (*Scyliorhinus canicula*)

This chapter is based on

Desender, M., Kajiura, S., Ampe, B., Dumolein, L., Polet, H., Chiers, K. and Decostere A. 2017. Pulse trawling: evaluating its impact on prey detection by small-spotted catshark (*Scyliorhinus canicula*). Journal of Experimental Marine Biology and Ecology 486; 336-343

Abstract

Pulse fishing may pose a promising alternative for diminishing the ecosystem effects of beam trawling. However, concerns about the impact on both target and non-target species still remain, amongst others the possible damage to the electro-receptor organs, the ampullae of Lorenzini, of elasmobranchs. The current study aimed to examine the role of pulsed direct current (PDC) used in pulse trawls on the electro-detection ability of the small-spotted catshark, *Scyliorhinus canicula*. The electroresponse of the sharks to an artificially created prey-simulating electric field was tested before and after exposure to the pulsed electric field used to catch flatfish and shrimp. No statistically significant differences were noted between control and exposed animals, both in terms of the number of sharks exhibiting an electroresponse prior to and following exposure as well as regarding the timing between onset of searching behaviour and biting at the prey simulating dipole. These results indicate that, under the laboratory circumstances as adopted in this study, the small-spotted catshark are still able to detect the bioelectric field of a prey following exposure to PDC used in pulse trawls. However, to fully grasp the impact of PDC on elasmobranchs, further studies are imperative, including examining the effect on reproduction and young life stages, the longer-term and indirect influences and experiments under field conditions.

Keywords: Pulse fishing, electrotrawling, ampullae of Lorenzini, small-spotted catshark, prey detection, beam trawl

6.1. Introduction

In the North Sea, 90% of all demersal fish, shell and crustacean landings are caught with bottom trawls (STECF, 2014a). However, this type of fishery elicits well-known disadvantages for the ecosystem including consuming high amounts of fuel, disturbing the seabed and producing high discard levels due to poor selectivity (Jennings and Kaiser, 1998; Lindeboom and De Groot, 1998; Kaiser et al., 2000; Paschen et al., 2000; Piet et al., 2000; Depestele et al., 2014; 2015). The landing obligation under the Common Fisheries Policy (CFP) (Council of the European Union, 2012) will be implemented stepwise between 2016-2019 for the demersal fisheries (STECF, 2014b; 2015). In order to meet the obligations imposed by the CFP and hence increase these fisheries' sustainability, electrical stimulation in fishing gear, beam trawls in particular, is considered a promising alternative resulting in reduced seabed disturbance, by-catch and fuel consumption and an increase in species selectivity (Boonstra and De Groot, 1974; Stewart, 1975; Polet et al., 2005b; Soetaert et al., 2015; van Marlen et al., 2014). Two major types of pulse gears may be discerned creating heterogeneous electric fields. Firstly, a high frequency, 45-80 Hz, bipolar pulse with a conductor voltage of 45-60V and pulsewidth of 100-270µs is used to provoke a cramp reaction in flatfish (Stewart, 1977; de Haan et al., 2016). Secondly, a low frequency, 5 Hz, unipolar pulse with a 60V conductor voltage and 500µs pulsewidth induces a tail-flip in shrimps forcing them to jump up out of the seabed (Verschueren and Polet, 2009; Verschueren et al., 2012; 2014). In spite of fishing by means of electricity being prohibited by the EU since 1998, a derogation for the southern North Sea was manifested in 2009 (EU, 1998). Currently, each member state may equip 5% or 10% (the Netherlands) of their beam trawl fleet with pulse gears (EU, 2009; 2013). Consequently, 83 electrotrawlers using the flatfish pulse, targeting in particular sole (*Solea solea*) and plaice (*Pleuronectes platessa*), are operating in the southern North Sea. In addition, 8 ships are equipped with the electrotrawl for catching brown shrimp (*Crangon crangon*) (Pers. comm. Bart Verschueren). In order to provide sufficient basis for dispensing with the standing ban completely and implement this fishing technique on a broad commercial scale, one should clarify possible adverse ecosystem effects in accordance with the principles of the precautionary approach and responsible fishing (FAO, 2011). Despite the spinal injury encountered in cod (de Haan et al., 2008; 2011; 2016; Rasenberg et al., 2013; van Marlen et al., 2014), results of various studies substantiate a tentatively positive attitude towards electrical fishing in terms of sustainability (Polet et al., 2005a; Smaal et al., 2005; van Marlen et al., 2009; Teal et al., 2014; Desender, 2016a; 2016b; Soetaert, 2014; 2016; de Haan et al., 2016). However, major gaps in knowledge on the impact of electrical fishing still remain. Since 2006 "The international council for the exploration of the sea" (ICES) has urged investigation of the possible effects of pulse trawling on electro sensitive elasmobranchs (sharks, rays and skates) (ICES, 2006 a; b). In response to this question, De Haan et al. (2009) exposed dogfishes to the flatfish pulse under laboratory conditions. Only weak responses were noted and no increased mortality, macroscopic lesions nor aberrant feeding behaviour were observed. Despite these reassuring results, this does not demonstrate that the electro-receptor organs, the ampullae of Lorenzini (AoL) are left undamaged as only dead fish pieces were provided as food. Indeed, benthic elasmobranchs especially rely highly upon their AoL to locate their prey buried in the seabed during the final moments of foraging (Dijkgraaf and Kalmijn, 1966; Kalmijn, 1971; Kajiura et al., 2010). Within close proximity, they can detect weak electric fields produced by living organisms, inanimate objects such as underwater electric cables, temperature gradients or the Earth's magnetic field. (Kalmijn, 1972; Paulin, 1995; Gill and Kimber, 2005). In addition, electroreception not only plays a role in prey detection but is also important in courtship and reproduction, predator avoidance, orientation to local inanimate electric fields and possibly geomagnetic navigation (Kalmijn, 1978; Tricas et al., 1995; 2004). The above leads to the research hypothesis that electrical signals generated by the pulse trawl may affect the AoL, hence impacting the elasmobranch's individual fitness. Therefore, the intention of the current study was to assess the effects of electric pulses, used in both flatfish and shrimp electrotrawling, on the functioning of the highly sensitive AoL. Small-spotted catsharks, formerly named lesser spotted dogfish (*Scyliorhinus canicula*), were employed as a representative for benthic electro sensitive elasmobranchs. For that purpose, the response towards an artificially created electric field, mimicking the bioelectric field emitted by their prey, was observed prior to and following exposure to the electric field generated by an electrotrawl.

6.2. Materials and Methods

6.2.1. Animals and housing

Fifty-three small-spotted catsharks were collected with beam trawls in the English Channel during commercial fishing practices and transported to the Institute of Agricultural and Fisheries Research (ILVO) in Ostend, Belgium. Eleven males and 42 females (52 +/- 7 cm total length) were acclimatized for a minimum of three weeks in a 4200L rectangular holding tank (140*600*60 cm) filled with aerated natural seawater and supplied with a mechanical and biological filter system. With regard to the water quality, the following values were recorded: 16°C temperature; 34‰ salinity; 8 pH; 7.5 dH; <25 mg/L nitrate, <0.2 mg/L nitrite, <0.1 mg/L ammonia. The photoperiod matched natural conditions. During the acclimatization period, each fish was fed twice a week with 20g (3% body weight) chopped whiting, herring, squids, shrimps or flatfish. The experimental protocol was approved by the ethical committee of ILVO (ID: 2012/171).

Seven 360L polyethylene behavioural arenas (110*70*60 cm) supplied with natural sea water were utilized. The tanks were arranged serially and connected to one trickling filter, with animals not being able to move between aquaria. Individuals were housed in 9 mixed sex (one male, two females), 8 single sex groups of three females and one group with two males. Three different colours of floy-tags, inserted cranially to the first dorsal fin, enabled individual identification on subsequent video recordings. Prior to an experimental trial, an additional acclimatisation period of one week was imposed following fish transfer, during which food was withheld. Thereafter, food rations were reduced to 13g (2% of bodyweight) per week during the experimental trials.

6.2.2. Experimental design

Each experimental trial was divided into two periods as described below (Figure 6.1). During the first period, the fish was allowed to acclimatize to the introduction of an acrylic plate (Figure 2) connected to an electrical prey simulator whereby the food response (consumption of food) was observed during four consecutive days. Thereafter, the animals were deprived of food for two days, after which the second period started with the recording of the elicited electroresponse (biting towards the prey dipole electric field). That same day the animal was exposed to an electric field used to catch shrimp or flatfish at sea (Figure 3). Following exposure, the electroresponse towards the prey simulator was again determined in its original behavioural arena during three consecutive days, with the first testing performed between 15 and 24h following exposure to the trawl electric field.

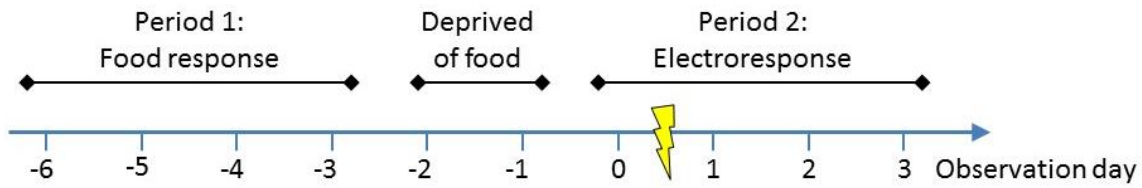


Figure 6.1: Overview of an experimental trial divided in two periods wherein the food and electroresponse are recorded during four consecutive observation days. Exposure to a pulse trawl electric field took place on day 0 (=).

Period 1: food response prior to exposure to the electric pulses

After unplugging of all pumps and electrical devices in and around the tanks to eliminate background electric fields, the acrylic plate connected to the prey simulating device was introduced. Five minutes following the introduction of the apparatus, the video camera was activated and one dipole was turned on, followed by the introduction of 1.3 g of chopped herring or whiting presented onto the active dipole. Once the food was consumed, the prey simulator was turned off. Once again, the simulator was switched on and off upon the provision and consumption of a new portion of food, respectively.

This process was repeated until each individual received a maximum of 2.6 g of herring or whiting per day, or after ten minutes following the introduction of the food, the latter being the case if the animals did not exhibit a feeding response. Following, the unconsumed food was removed and the video camera and prey simulator were turned off and moved to the next randomly chosen experimental arena. Behavioural observations included i) the reaction time between food introduction and initiation of foraging behaviour, characterised by increased swimming activity and S-shaped turning close to the bottom (Kajiura and Holland 2002; Kimber et al. 2009), and ii) the time to first feeding. These parameters were recorded during four consecutive days for each individual. The difference between these two parameters provided the delay time to elicit a bite response towards the provided food.

Period 2: exposure to the electric pulse field and electroresponse

The second period was initiated with the testing of the electroresponse of each individual towards the prey simulating dipole. For that purpose, the experimental procedure as described above was repeated, except that when the dipole was turned on, the foraging behaviour was invoked by the introduction of 20ml of whiting juice through the odour delivery tube and no food was introduced. Once a particular shark had bitten at the prey simulating dipole, the dipole was turned off. The dipole electrical stimulus was turned on again when another shark entered the activity zone (10 cm radius circles centered around the dipole (Kimber et al., 2009)). The reaction time following scent introduction to exhibit food searching behaviour and to bite towards the dipole, were determined. The difference between these two parameters resulted in the delay time to bite towards the prey stimulus. Behaviour was monitored until all animals of one group had bitten the prey simulating electrode or ten minutes after the introduction of food derived scent should no bite response towards the dipole have occurred. Before moving the video camera and prey simulator to the next randomly chosen arena, each shark received 2.6 g of chopped whiting or herring presented on the active dipole.

Following the evaluation of the first electroresponse for all sharks during one experimental trial, the animals were individually transferred to a treatment tank of 300 L (110*70*45 cm) where they were subsequently exposed to an electric pulse field used to catch brown shrimp or flatfish. Sharks were orientated perpendicularly between two electrodes, opposite the middle of the conducting elements, during exposure. They were positioned in a polyethylene netting (58 cm, 15 cm diameter, 1 cm^2 mesh size) with a cylindrical profile. Following transfer to the treatment tank, the pulse trawl generator was directly switched on during an exposure period of 5 seconds. The behaviour during exposure was observed.

In total 15 sharks were randomly exposed to the pulse to catch brown shrimp and 8 to the flatfish pulse. In addition, 30 controls were included and treated similarly, except for the exposure to the electric field. After the exposure, the animal was released back in its original behavioural arena.
The following three consecutive days the electro response towards a prey stimulus subsequent to the scent introduction was observed as described above. The delay time to bite towards the dipole was calculated by subtracting the time following scent introduction to display food searching behaviour from the time to first bite towards the dipole.

6.2.3. Experimental equipment:

The prey simulator:

A 9V battery-powered generator (Kajiura and Holland, 2002) was employed to deliver a direct current (DC) prey simulating electric field. The stimulus source supplied current (9 µA) via an underwater cable tightly sealed onto two seawater filled aquarium tubes (50 cm length, 3 mm internal diameter). The open ends of these salt bridges were attached through pre-drilled holes on a transparent acrylic plate (100*50*0.5 cm). The holes were spaced 1 cm apart, creating a dipole electric field that mimicked the size of naturally occurring prey. The acrylic plate was equipped with two dipoles, with the dipole centers spaced 35 cm apart (Figure 6.2). A multimeter in series enabled monitoring of the current being applied between the electrodes of the active dipole. During each trial, only one of the dipoles was energized while the other dipole functioned as a control. An odour delivery polyethylene tube was also inserted into the center of one half of the acrylic plate from below, at 17.5 cm distance from both dipoles (Figure 6.2). A syringe was used to introduce an odourant into the water surrounding the electrode array. The food derived scent consisted of a 20 ml seawater solution containing sieved whiting and squid rinse. The odour stimulus was required to invoke foraging behaviour and attract the sharks towards the electric dipole. All tubing and connectors were shielded underneath the acrylic plate. Bricks arranged around the edges of the base prevented the acrylic plate from floating. The equipment was easily transferred between the seven experimental arenas wherein the dipole was randomly positioned.

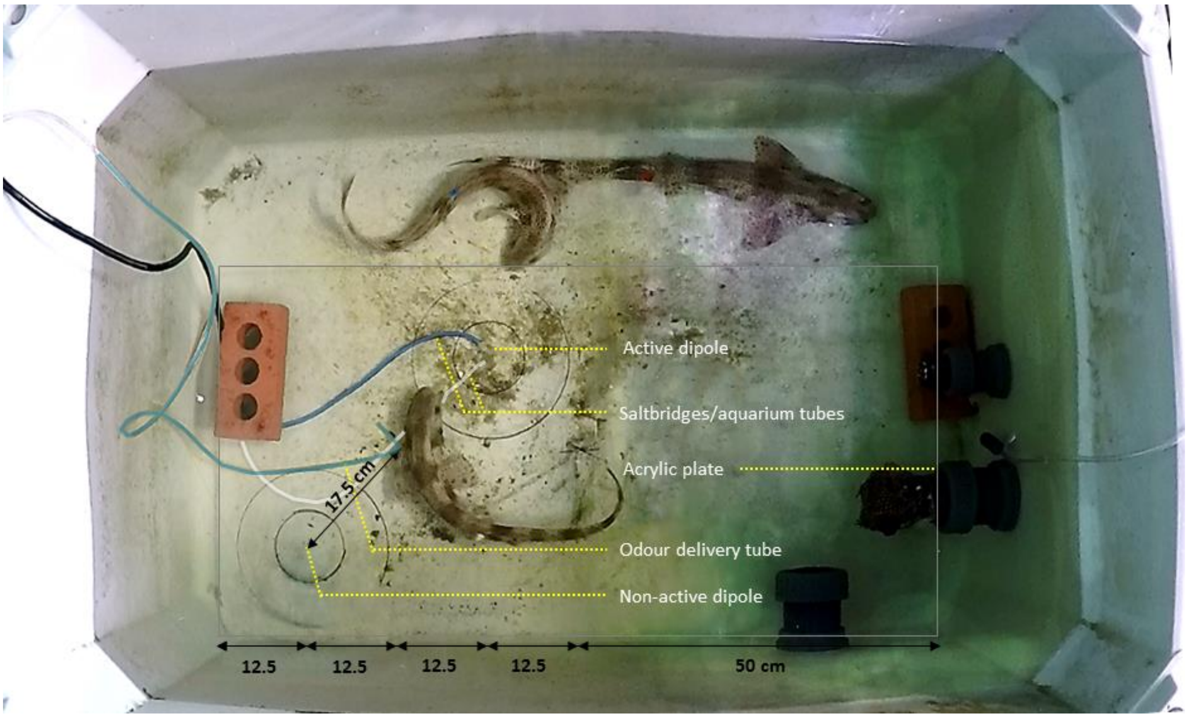


Figure 6.2: Acrylic plate (100*50 cm) equipped with two dipoles spaced 35 cm from each other with the odour delivery tube attached in the middle between them at 17.5 cm from both dipoles.

Generating the shrimp and flatfish pulse trawl electric field:

To generate the same heterogeneous pulsed DC electric field used to catch brown shrimp at sea, the exposure aquarium was equipped with two 50 cm long threadlike electrodes of 1.2 cm diameter, placed on the bottom of the aquarium (Figure 6.3A). Each electrode had a diameter of 12 mm and was composed of six stainless steel strands on the outside and a central solid copper strand inside. These conductors were placed in parallel at a distance of 65 cm and were electrically connected with an adjustable laboratory pulse generator (LPG, EPLG bvba, Belgium). Pulse parameter settings in the LPG were characterized by a unipolar square pulse shape and pulse duration of 500 µs generated at a frequency of 5 Hz, consequently building up an electric pulse field with an interval of 200 ms. The applied voltage to the electrodes had a constant amplitude of 60 V (Verschueren and Polet 2009, Verschueren et al., 2012).

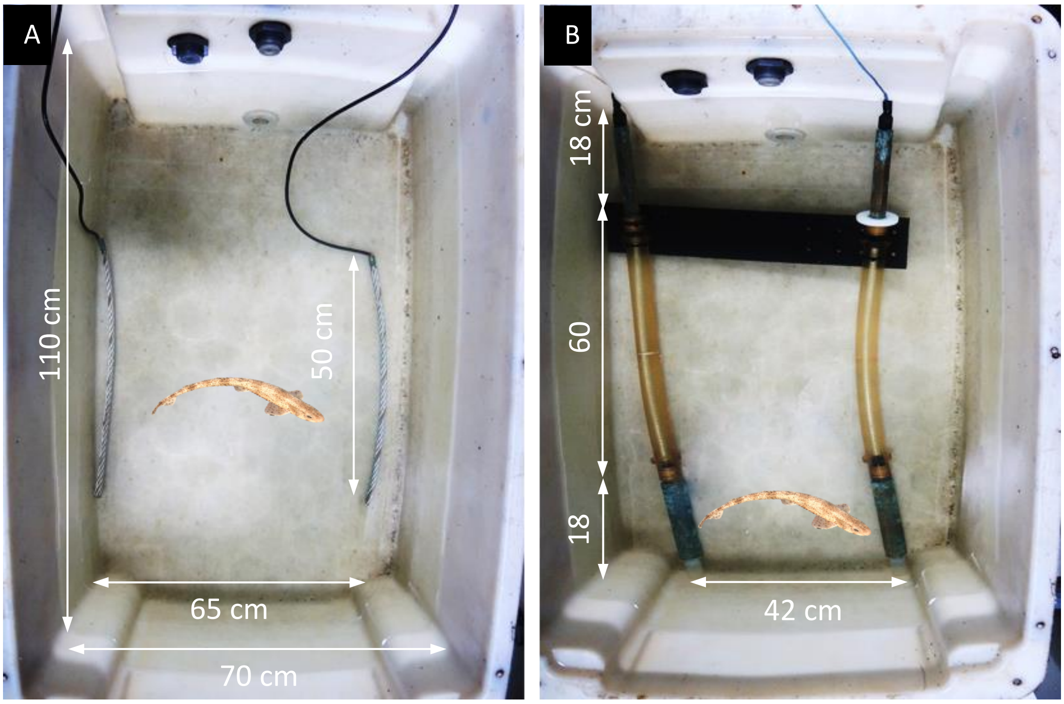


Figure 6.3: A) Two 50 cm long electrodes spaced 65 cm apart used in electrotrawling for brown shrimp B) Two electrodes spaced 42 cm apart used to catch flatfish. Each electrode was implemented with two conductors of 18 cm and an isolated extension of 60 cm.

To simulate the heterogeneous electric field used to catch flatfish, two electrodes of 96cm were adopted (de Haan et al., 2009). Each electrode was implemented with two conductors of 18cm (32 mm diameter) with an isolated extension of 60 cm between both (Figure 6.3B). The distance between electrodes was set to 42 cm. Pulse parameter settings generated in the LPG were characterized by a bipolar square pulse shape and pulse duration of 250 µs generated at a frequency of 80 Hz. The applied voltage to the electrodes had a constant amplitude of 60 V (Soetaert et al., 2016a).

Pulse characteristics were closely monitored using a Tektronix® Oscilloscope type TDS 1001B.

6.2.4. Data Analysis

A generalized linear mixed model (glmer function in R 3.2.2, R Foundation for Statistical Computing, Vienna, Austria) was fitted to the data using treatment (control or exposed to shrimp or flatfish pulse), time and their interaction as categorical fixed effects and the animal as random effect. A binomial distribution with a logit-link function was used to compare the presence or absence of a food or electroresponse between control and exposed individuals during period one and two respectively. The differences in the evolution of the response before and after exposure between control and exposed were tested using a post-hoc linear contrast. Kaplan Meier plots were generated to visualize the electroresponse following exposure.

The delay time to bite towards the food or towards a prey simulating electric field was analysed with a similar linear-mixed effects model. The differences in the evolution of the delayed time to bite before and after exposure between control and exposed were tested using a post-hoc linear contrast. The analysed data were considered sufficiently normally distributed, based on the graphical evaluation (histogram and QQ-plot) of the residuals.

6.3. Results

No dead fish nor macroscopic injuries were observed throughout the whole experimental trial. Representative data regarding food and electro response for period 1 and 2, respectively, are listed in Table 6.1.

Period 1: food response prior to the exposure to the electric pulses

Not all individuals exhibited searching behaviour following the introduction of food. Indeed, 6 days prior to exposure only 62% (31/50) commenced foraging and consequently took the food presented on the active dipole (Table 6.1). Three days before exposure, this number had increased to 71% (38/53). Over the four day observation period: 4 animals never ate, 6, 10 and 11 individuals consumed food on one, two or three days, respectively and 22 animals displayed a food response every day. Searching behaviour started 99 ± 121 s after the food was provided. The delay time to elicit a bite response towards the provided food over the four day observation period prior to exposure to electric pulses was 79 ± 94 s (Figure 6.4).

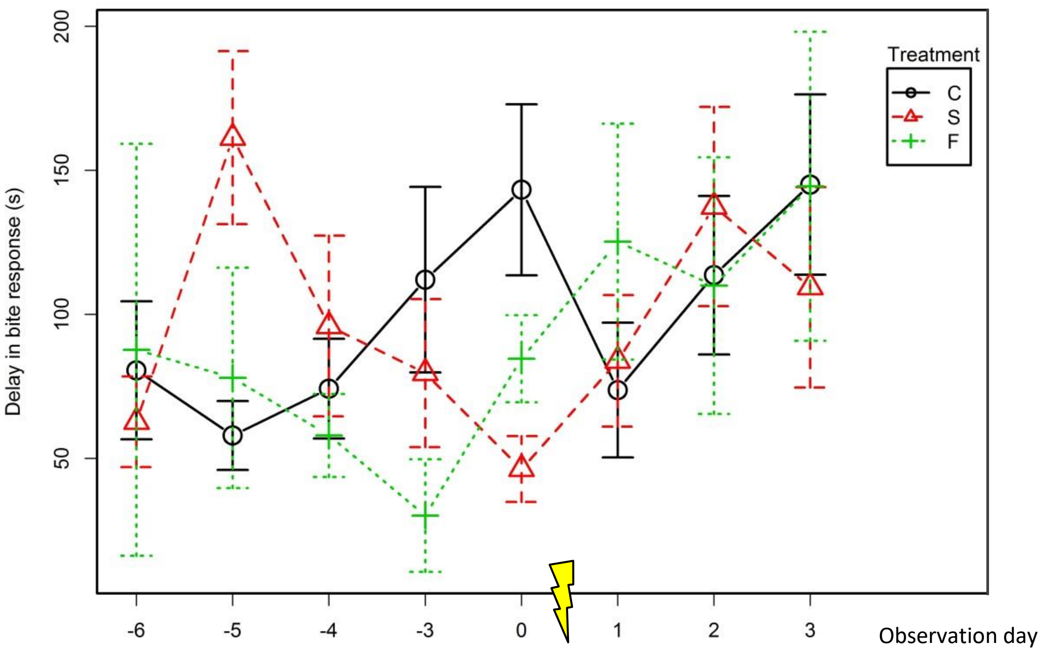


Figure 6.4: The delayed time to elicit a bite response towards a food source or towards a prey simulated dipole for responding control (=C) and responding exposed (S= 5 Hz shrimp pulse; F= 80 Hz flatfish pulse) sharks per day. The treatment was given on day null, after testing of the electroresponse.

Period 2: exposure to the electric pulse field and electroresponse

Following two days of feed deprivation and before exposure to the electric pulse field, the electroresponse amounted to 77%, whereby 41 out of 53 individuals bit the prey stimulus (Table 6.1). Initiation of searching behaviour started at 57 ± 71 s following scent introduction and the delay time to bite towards the dipole electric field was 105 ± 111 s (Table 6.1; Figure 6.4).

During the exposure to both the shrimp as well as the flatfish pulse, all sharks displayed a cramp reaction which made the fish motionless during the 5 second pulse period. Simultaneously, the eyes closed. The control animals displayed active swimming behaviour in the net. On day one after exposure, following the introduction of a food-derived scent, an electroresponse was demonstrated in 66% (20/30), 73% (11/15) and 88% (7/8) of control and shrimp or flatfish exposed fish, respectively (Table 6.1; Figure 6.5). Three days after treatment, 80% (24/30) of control animals and 87% (13/15) and 88% (7/8) of animals being exposed to the shrimp and flatfish pulse, respectively, bite at least once towards the prey simulating dipole (Figure 6.5). Eight out of the nine sharks that never elicited an electro response following treatment (ID nr. 9, 11, 12, 29, 39, 45, 47, 52) did not display food searching behaviour (Table 6.2). The three not biting exposed animals (ID nr. 9, 11, 47) also exhibited a poor food response before treatment. Indeed, one animal came to feed two times and 2 sharks fed only once over the whole monitoring period. One control treatment shark (ID nr. 33) demonstrated food searching behaviour but did not bite. This behaviour, displaying food searching behaviour but not biting at the dipole, was sporadically observed sixteen times during the whole monitoring period in 10 controls and 5 exposed animals (ID nr. 1, 11, 24, 25, 15, 17, 30, 33, 36, 38, 41, 46, 48, 51, 52) (Table 6.2). No significant change of abnormal food or electroresponse behaviour could be distinguished in shrimp ($p=0.222$) or flatfish pulsed ($p=0.925$) animals compared to their control groups before or after exposure. The second, not active control dipole was never bitten, excluding interference from olfaction and visibility of the electrodes. The reaction time between scent introduction and initiation of foraging behaviour was 68 ± 87 s. The delay time to bite between onset of foraging behaviour and the actual bite towards the simulated prey totalled on average 114 ± 102 s over the three day observation period after treatment and was not significantly influenced by being exposed to the shrimp (p=0.1315) or flatfish pulse (p=0.0998) relative to the control groups before or after exposure (Figure 6.4).

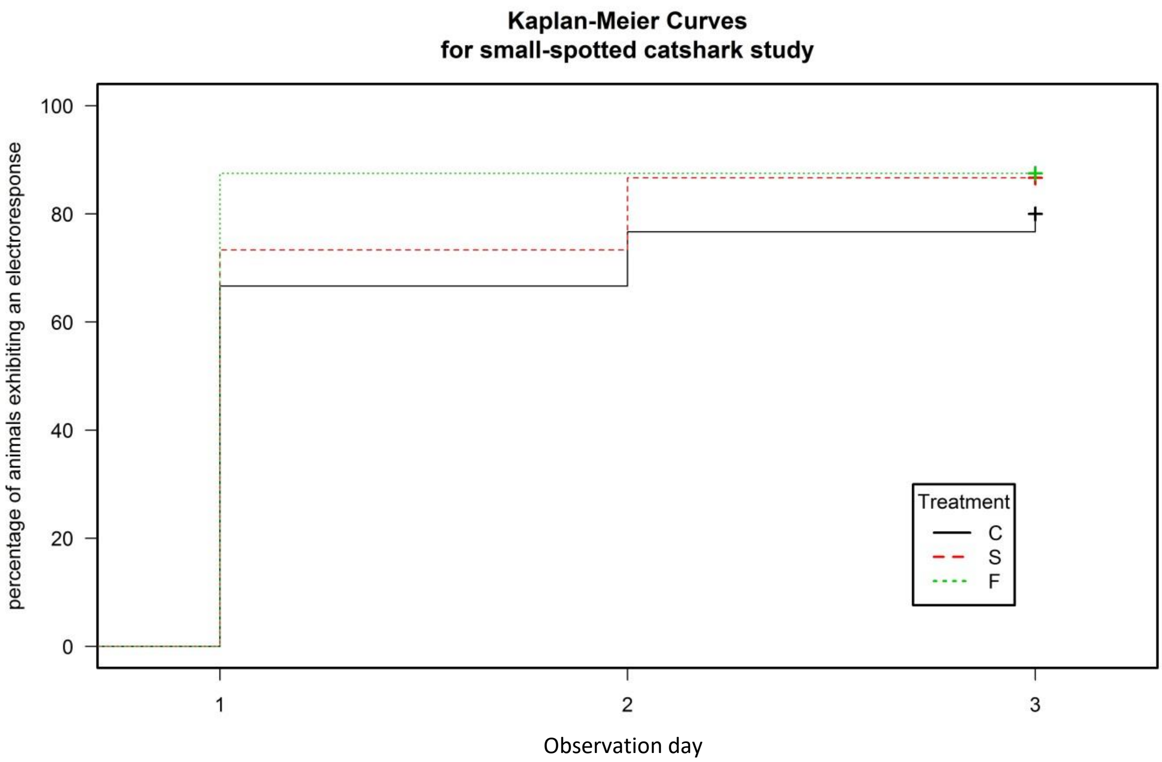


Figure 6.5: Kaplan-Meier survival plot representing the percentage of animals exhibiting an electroresponse after one, two and three days following treatment. C= control; S= exposure to the 5 Hz shrimp pulse; F=exposure to the 80 Hz flatfish pulse.

Table 6.1: Data on the activity and food - or electroresponse behaviour. The treatment was given after testing of the first electroresponse on day 0. (C= control; S= exposure to the 5 Hz shrimp pulse; F=exposure to the 80 Hz flatfish pulse.)

				Food response																Electroresponse															
				Day -6				Day -5				Day -4				Day -3				Day 0				Day 1				Day 2				Day 3			
Treatment	Length (cm)	Maximum # of animals	# Female	# Animals	# Active	# Bite	Delay time (s)	# Animals	# Active	# Bite	Delay time (s)	# Animals	# Active	# Bite	Delay time (s)	# Animals	# Active	# Bite	Delay time (s)	# Animals	# Active	# Bite	Delay time (s)	# Animals	# Active	# Bite	Delay time (s)	# Animals	# Active	# Bite	Delay time	# Animals	# Active	# Bite	Delay time (s)
C	51.5±7.2	30	24	27	15	15	81±93	30	18	17	48±46	30	22	22	74±81	30	25	22	97±139	30	24	21	143±139	30	22	20	74±105	30	17	15	119±111	23	12	12	145±108
S	54.2±5.9	15	12	15	12	12	63±54	15	15	14	137±109	15	13	12	96±113	15	11	11	65±76	15	14	12	47±34	15	11	11	84±76	15	12	12	126±116	8	7	7	109±92
F	51.8±7.9	8	6	8	4	4	88±143	8	7	7	56±80	8	5	5	58±32	8	5	5	30±44	8	8	8	85±43	8	7	7	65±108	8	6	5	110±100	8	5	4	144±107
Total	52.3±6.9	53	42	50	31	31	75±85	53	40	38	80±88	53	40	39	79±88	53	41	38	80±116	53	46	41	105±111	53	40	38	86±97	53	35	32	120±108	39	24	23	134±100

Table 6.2: Individual data on the activity and food-or electroresponse behaviour. (NT = not tested; Red = absence of an electroresponse; Yellow= active food searching behaviour present but food or electro-response absent; C= control; S= exposure to the 5 Hz shrimp pulse; F=exposure to the 80 Hz flatfish pulse.)

			Food response																Electroresponse								
			Day -6			Day -5			Day -4			Day -3			Day 0				Day 1			Day 2			Day 3		
ID	Length; cm	Sex	Active = 1	Bite = 1	Delay time (s)	Active = 1	Bite = 1	Delay time (s)	Active = 1	Bite = 1	Delay time (s)	Active = 1	Bite = 1	Delay time (s)	Active = 1	Bite = 1	Delay time (s)	TREATM.	Active = 1	Bite = 1	Delay time (s)	Active = 1	Bite = 1	Delay time (s)	Active = 1	Bite = 1	Delay time (s)
1	55	F	0	0	NT	1	1	71	0	0	NT	1	0	NT	1	1	09	C	0	0	NT	1	1	NT	NT	NT	NT
9	51	M	0	0	NT	1	1	229	0	0	NT	0	0	NT	1	1	34	S	0	0	NT	0	0	NT	NT	NT	NT
11	58	F	0	0	NT	1	0		0	0	NT	0	0	NT	1	1	69	S	0	0	NT	0	0	NT	NT	NT	NT
12	59	F	NT	NT	NT	1	1	01	1	1	55	0	0	NT	1	1	30	C	0	0	NT	0	0	NT	NT	NT	NT
24	50	F	1	1	24	1	1	28	1	1	16	1	1	12	1	1	33	C	1	1	210	1	0	NT	1	1	238
25	56	F	1	1	54	1	1	45	1	1	23	1	1	5	1	1	41	C	1	1	196	1	0	NT	0	0	NT
15	56	F	1	1	8	1	1	0	1	1	96	1	1	199	1	0	NT	S	1	1	164	1	1	04:04	0	0	NT
17	56	F	1	1	71	1	1	0	0	0	NT	1	1	20	1	0	NT	C	1	1	3	0	0	NT	0	0	NT
29	59	F	0	0	NT	0	0	NT	0	0	NT	0	0	NT	0	0	NT	C	0	0	NT	0	0	NT	0	0	NT
30	65	F	1	1	26	1	1	30	1	0	NT	1	1	00:00	1	1	66	S	1	1	115	1	1	131	1	1	197
33	52	F	1	1	12	1	1	79	1	1	32	1	1	34	1	1	95	C	1	0	NT	0	0	NT	0	0	NT
36	47	F	0	0	NT	1	1	0	1	1	53	1	1	16	1	1	77	F	1	1	77	1	0	NT	1	1	302
38	47	F	1	1	367	1	1	0	1	1	158	1	0	NT	1	1	111	C	1	0	NT	0	0	NT	1	1	394
39	66	F	0	0	NT	0	0	NT	0	0	NT	0	0	NT	0	0	NT	C	0	0	NT	0	0	NT	0	0	NT
41	43	M	0	0	NT	1	1	207	1	1	90	0	0	NT	1	1	75	F	1	1	358	1	1	228	1	0	NT
45	48	M	1	1	185	0	0	NT	1	1	6	1	1	05:05	1	1	00:09	C	0	0		0	0		0	0	NT
46	55	F	0	0	NT	0	0	NT	1	1	134	1	1	298	1	0	NT	C	1	1	67	1	1	54	1	1	31
47	60	F	0	0	NT	0	0	NT	0	0	NT	0	0	NT	1	1	27	F	0	0		0	0	NT	0	0	NT
48	39	M	0	0	NT	0	0	NT	1	1	341	1	1	10	1	0	NT	C	1	1	4	1	1	116	1	1	161
51	60	F	0	0	NT	1	0	NT	1	1	199	1	1	15	1	1	273	C	1	1	428	0	0	NT	1	1	139
52	39	F	0	0	NT	0	0	NT	1	1	41	1	0	NT	0	0	NT	C	0	0	NT	0	0	NT	0	0	NT

6.4. Discussion

Elasmobranchs are affected by high bycatch rates and tend to exhibit a K-selected life history strategy which makes them especially vulnerable (White et al., 2012). They have become a focus for marine conservation action due to fishery driven global declines in many elasmobranch populations (Molina and Cooke 2012; Jordan et al., 2013; Kynoch et al., 2015). Therefore, disturbing individual fish may have a serious impact on population levels and consequently top down effects through trophic cascades (Baum and Worm, 2009). There is a growing concern that these vulnerable fish may be affected by increasing occurrences of anthropogenic electrical sources in many of the world's coastal, benthic habitats (Gill and Kimber, 2005; Normandeau et al., 2011). In response to questions put forward by ICES regarding the effect of pulse stimulation in commercial beam trawling on components of the marine ecosystem, the present study was conducted. These engendered data, as well as the results from the study undertaken by de Haan et al. (2009), did not reveal macroscopic injuries nor death as a result of exposure to the electric pulse fields. The research group of de Haan et al., (2009) additionally did not note aberrant feeding behaviour in the 14d observation period following exposure to the flatfish pulse. However, it needs to be kept in mind that in captivity sharks may easily find their daily chopped meal in the clean survival tanks without having to resort to their electro sensitive AoL. This is not the case in their natural habitat where these fish fully depend on their electro sensitive organs to detect the electric field surrounding the prey burrowed in the seabed (Tricas and Sisneros, 2004). To our knowledge, this is the first study to examine the impact of PDC used in pulse trawls on the electro-detection ability of an elasmobranch. Small spotted catsharks were used as a model organism in the current study. Although this species encompasses rather robust animals with less conservation issues, sensitivity to electric field strengths may be regarded as similar across species (Kajiura and Holland, 2002; McGowan and Kajiura, 2009; Jordan et al., 2011; Jordan et al., 2013). In addition, coastal species and those feeding on benthic prey, such as *S. canicula*, are most likely to rely heavily on the electrosensory system, warranting their inclusion as a model species in the current study (Tricas and Sisneros, 2004; Kajiura et al., 2010).

In our study, no statistically significant differences were noted between control and exposed animals, both in terms of number of sharks exhibiting an electroresponse to the dipoles prior to and following exposure, as well as regarding the delay time to bite to the prey simulating dipole. Nine animals, that is 6 controls (20%), 2 (13%) shrimp - and 1 (13%) flatfish pulsed, never bit the dipole electric field after treatment, with 8 of these sharks not displaying active food searching behaviour. These animals laid on the bottom of the tank and did not move during the 10 minute behavioural recordings. As a result, they did not encounter the DC dipole fields. Up to 25% of non-responding sharks were encountered in other studies as well (Filer et al., 2008). This urges us to speculate that the failure to initiate a bite response may therefore not be rooted in the inability to detect the stimulus but because sharks were not hungry enough to be motivated to search for food. As motivational state changes between feeding, fasting, and refeeding, the best response, 79%, was noticed on day 0. This is the day following two days of food deprivation and before exposure to the electric pulse field. The lowest response, 58%, was observed on day 3 after exposure to the electric pulse field, the last day of the trial when the sharks had been fed on the preceding days.

In the present study only a single type of prey simulating electrical stimulus was tested. Sharks are able to detect and respond towards a variety of electric fields such as fields of conspecifics to find a suitable mate (Tricas and Sisneros, 1995), prey (Kalmijn 1972, Bedore & Kajiura 2013), and predators (Sisneros et al., 1998; Kempster et al., 2013). They are also suspected to be able to detect electric fields induced by their movement with respect to the earth's magnetic field (Kalmijn 1974; 2000; Paulin 1995) and geomagnetic anomalies (Klimley 1993; Montgomery and Walker, 2001). The prey-simulating electric field chosen for the experiment is within the range shown to be attractive to catshark and comparable to those produced by a variety of species commonly found in their opportunistic diet (Kalmijn 1971, 1972; Kajiura et al., 2002; Filer et al., 2008; Kimber et al., 2009; 2011; 2013). Different species emit varying and complex DC and AC bioelectric fields. Prey-type DC electric fields have a magnitude of, e.g.

39µV up to 500µV for teleosts or up to 50µV for crustaceans (Kalmijn 1972; Bedore and Kajiura, 2013). According to Kalmijn (1972) and Haine et al. (2001), but in contrast with Bedore and Kajiura (2013) each species' field increases in strength with increasing specimen size. Furthermore, if an organism is injured, the DC electric field may dramatically rise up to more than 1250 µV for crustaceans (Kalmijn, 1972; 1974). Small-spotted catsharks attracted to fields around 0.1 – 1.5 µV cm^{-1} (Yano et al., 2000; Tricas, 2001; Kimber et al., 2011) and detection thresholds up to 5-20 nV cm^{-1} were observed (Dijkgraaf and Kalmijn; 1966; Peters and Evers 1985; Tricas and New, 1998). These animals are able to distinguish different types of electric fields with a clear preference for higher magnitude electric fields of 9 or 90µA compared to 0.9µA (Kimber et al., 2011). However, when the current would increase much beyond 100 µA (Kraus and Fleisch, 1999) or when electric fields of 4-10 µV cm^{-1} would be presented, catsharks are expected to avoid these fields (Gill and Taylor, 2001; Gill et al., 2014). As catsharks seem to be unable to discriminate between or show no preference for artificial and natural fields of a similar magnitude (Kimber et al., 2011), this may have implications when considering possible interactions with anthropogenic electric fields such as underwater power cables (Gill and Taylor, 2001) or indeed pulse trawls. The latter should theoretically repel elasmobranchs away rather than attract them as the electric field of an electrotrawl is at least 30 V m^{-1} when measured in the middle of two electrodes. This is at least 10,000 times higher than the 10 µV cm^{-1} that causes avoidance behaviour in sharks. According to Gill and Taylor (2001), an external uniform field of 1000 µV m^{-1} is reduced to 1µV m^{-1} over a distance of 100 m. However, as the high frequency (45-80Hz) electric field of the pulse trawl used to catch flatfish is outside the detection limits of electroreceptive organisms (<16Hz) (Kalmijn, 1972; Tricas and New, 1998) only the low frequency (5Hz) pulse trawl used to chase brown shrimp might be detectable by elasmobranchs. In the supposition that sharks, skates or rays sense this pulse trawl and in case it may be assumed that this results in avoidance behaviour, one might speculate that bycatch rates of elasmobranchs hence may be reduced. However, small spotted catsharks may be incapable of out-swimming an on-coming bottom trawl (Kynoch et al., 2015). That is almost certainly the case for skates that often bury into the seafloor. In case no avoidance behaviour is manifested in field situations and the dogfish consequently get caught in the electric field of the pulse trawl, the animals may become entangled in the top panel of the pulse trawl. Indeed, a common behavioural response following exposure was to accelerate upwards when exposed less than 0.1 m distance from an electrode used in flatfish pulse trawling (de Haan et al., 2009). By-catch data of beam and pulse trawls might give more information on possible escape behaviour of elasmobranchs and rectify or disprove the above.

With regard to assessing the impact of electric pulses on elasmobranchs, in addition to investigating the effect on the AoL, various other items need to be addressed. The research group of de Haan (2009) observed that all exposed groups produced eggs in a period of 7 months following exposure. However, effects of pulse trawling on the reproduction and development of younger life stages remains uncertain. Furthermore, no long-term studies have yet been conducted nor have possible other side-effects not measured in the present study such as stress, immune system impairment or behavioural alterations been examined. In addition, one needs to keep in mind that the current study was conducted under laboratory conditions which do not take into account the variable and dynamic character of the marine environment in which various parameters may change quickly at a specific site within a short time period. This renders field experiments imperative in which, as stated above, by-catch data for elasmobranchs are collected and behavioural alterations monitored.

6.5. Conclusion

The present study is the first to tackle the possible adverse effects of electrotrawls on vulnerable elasmobranchs and their electrosense organ involved in prey detection. Under the circumstances as adopted in this study, no altered foraging behaviour towards an electrically simulated prey was observed following exposure towards an electric field used in shrimp and flatfish electrotrawls.

Acknowledgments

The research leading to these results has received funding and support from the Agency for Innovation by Science and Technology in Flanders (IWT) and The European fisheries fund (EFF). We thank the Fishing Gear Technology group of the Flemish Scientific Institute for Agricultural and Fisheries Research (ILVO) for logistics and supply of the lab animals

CHAPTER 7: GENERAL DISCUSSION

7 General Discussion

In what follows, in first instance, the reasoning behind the various experimental set-ups is discussed in terms of selected fish species and life stage, electric field and orientation and the parameters for impact assessment. Consequently, a brief outline of the performed research is given, including the studies of the current PhD manuscript, followed by a critical appraisal of their actual significance in terms of assessing the impact of pulse trawling on marine aquatic organisms. To end, ideas for future research are elaborated upon.

7.1 Rationale for the adopted experimental set-up

The impact of the electric pulse fields was assessed in various fish species using different experimental set-ups and a multidisciplinary approach to investigate the following parameters (depending on the life stages and trial): mortality, behaviour (movements, prey capture), development, growth, macroscopic and microscopic lesions and spinal injury. Table 7.1 provides a survey of the performed experiments in the current manuscript listing the fish species, life stage, adopted pulse, electric field and orientation and the parameters used to evaluate the impact.

Table 7.1: Overview of performed experiments to give answers to the questions concerning the impact of electrotrawling

Chapter	Species	Life stage	Pulse	E-Field	Orientation	Parameters for impact assessment
3	sole, plaice, bull-rout, armed bullhead, cod	adult	shrimp	heterogeneous	random	survival, RX, macroscopic and microscopic lesions, behavioural alterations
4	cod	embryo, larva, juvenile	shrimp	homogeneous	random	survival, hatching, development, morphometrics
5	sole	embryo, larva	shrimp	homogeneous	random	survival, development, morphometrics
6	dogfish	adult	shrimp & flatfish	heterogeneous	perpendicular	prey capture

7.1.1: Selection of representative marine species

Electrotrawling will mainly have a possible negative effect on benthic marine species inside the trawl path. The electric field will indeed quickly drop to zero once outside the trawl path (Verschueren and Polet, 2009). Therefore, a founded selection of adult marine teleosts, inhabiting shrimp fishery areas and often present in the **bycatch** of brown shrimp trawlers, was subjected to low frequency electric pulses in Chapter 3. The species investigated were the economically important sole (*Solea solea*), plaice (*Pleuronectes platessa*) and cod (*Gadus morhua*). Cod has already proven to be vulnerable to electric pulses witnessed by the 7% spinal injury in commercial catches (de Haan et al., 2008; 2011; van Marlen et al., 2014; de Haan et al., 2016). Also discards such as bull-rout (*Myoxocephalus Scorpius*) and armed bullhead (*Agonus cataphractus*) were included in the study. The experimental animals were caught in the wild on board shrimp trawlers (except cod) and kept in captivity for several weeks. Only organisms that were in good condition (no major external injuries, barotrauma, normal movement of gill opercula, no impaired equilibrium or reaction to stimuli) were selected for the experiments. Due to the difficulties in capturing a sufficient number of cod and the low survival of these wild caught top predators, our studies included cultured cod from a Norwegian research facility.

For the studies in Chapter 4 and 5 on the impact of low frequency electric pulses on **young life stages**, a first prerequisite for the selected fish species was that it could be cultured from embryo to juvenile. Secondly, the species involved should have important spawning or nursery grounds in the North Sea. Taking both criteria into account, we chose two economically valuable species, **cod and sole**. Cod was chosen as a model organism for marine cold water round fish species. Sole was opted for as a model organism for bottom dwelling flatfish species. The latter undergoes a remarkable metamorphosis during development. Cod was cultured and weaned in the cod breeding centre (NOFIMA, Tromso, Norway). Sole eggs were delivered by the Institute for Marine Resources and Ecosystem Studies (IMARES, the Netherlands) and cultured at ILVO.

Electro sensitive elasmobranchs, more specifically sharks, were investigated in Chapter 6. These cartilaginous fish are often top predators in the ecosystem and tend to have K-selected life-history characteristics. Slow growth rates, late maturity and small litter render them particularly vulnerable to overexploitation. Numbers of elasmobranchs are declining worldwide with some species seriously depleted. Besides a shift in pulse fishing effort distribution was observed towards the western part of the southern North Sea, the area where the abundance of elasmobranchs is relatively high (Turenhout et al., 2016). Furthermore they use electro sensitive organs, ampullae of Lorenzini (AoL) to catch prey in the final moments of foraging. Catsharks are often used as a model species and are easy to house in captivity, justifying their selection in our studies. Furthermore, their populations are stable or increasing. In addition, their prey capture behaviour and role of the AoL has already been investigated in detail for decades (Kalmijn, 1981; Kimber et al., 2011).

7.1.2: Types of electrotrawls

At first focus was set on electro trawling for brown shrimp and the low-frequency 5Hz pulse as almost no information concerning this pulse was available. Additionally, and due to the innovative character of the experiments as described in Chapter 6, also the flatfish pulse was included. Nominal settings applied in the pulse generator and electrode design for both pulses are summarized in Table 7.2.

Table 7.2: Nominal settings for shrimp and flatfish pulse

	Electric field	Volt	Frequency	Pulse width	Electrode design	Electrode distance
Shrimp pulse	Heterogeneous	60 V	5 Hz	500 µs	12mm diameter 50-150 cm length	65-70 cm
	Homogeneous 150 V/m	36 V	5 Hz	500 µs	32*23*0.4 cm	24.5 cm
Flatfish pulse	Heterogeneous	60 V	80 Hz	250 µs	32 mm diameter 96 cm length (2 conductors of 18 cm and 1 isolator 60 cm)	42 cm

7.1.3: Adopted electric fields

Two kinds of exposures, homogeneous or heterogeneous, were used in the studies of this PhD research (Table 7.2). Adult fish were exposed in a **heterogeneous** electric field. In Chapter 3 and 6 this was created by using three or two thread shaped electrodes respectively. In Chapter 6, fish were orientated perpendicular and in the middle between electrodes by using a wire mesh cage. In this way the highest head-to-tail voltage was set over the fish body approaching a worst-case scenario. To improve the comparability with the situation in the field, three electrodes were used in Chapter 3. In this way the switch of the electric field between successive electrode pairs was mimicked. In this chapter also behavioural alterations were observed during and after the exposure with no interference by the fish's position or orientation during the experiment.

Due to their much more smaller size, and to exclude large differences in field strength, a **homogeneous** set up was chosen for the younger life stages exposed in Chapter 4 and 5. Life stages were randomly orientated in an exposure chamber between plate shaped electrodes. With a Voltage of 36 V applied onto plate shaped electrodes spaced 24.5 cm apart, a field strength of approximately 150V/m was created. This worst-case scenario simulates a small part of the heterogeneous electric field at 5 cm distance of a thread shaped electrode used at sea.

7.2. Impact of pulse trawling on marine aquatic organisms

7.2.1. What is known hitherto: a brief outline blending our studies and the existing literature

Electrotrawling is conquering the North Sea to a growing extent. Despite all the promising sustainable improvements, Europe understandably is keeping its precautionary approach. Indeed, knowledge on the impact of electric pulses on target and non-target species is limited. However, since 2008 research is ongoing and gaps in knowledge are diminishing.

No adult fish subjected to the **shrimp pulse** died during the monitoring period. Indeed, in Chapter 3 sole, plaice, armed bullhead, cod and pogge, and in Chapter 6 catsharks were exposed for 5 seconds and observed for 24 hours during which no mortality was noted. Also Soetaert et al. (2016a) observed no mortality 14 days after exposure in sole and cod. In earlier **survival** tests (Polet et al., 2005a) a 15 seconds lasting exposure was applied to the previously mentioned species and also dab (*Limanda limanda*), turbot (*Psetta maxima*), dragonet (*Callionymus* spp.), five-beard rockling (*Ciliata mustela*) and gobies (*Pomatoschistus* spp.) were included. After 10-30 days no effect on survival nor feeding activity was noted (Polet et al., 2005). Also in Chapter 3 the **behaviour** of species was observed in detail 30 min before and after the pulse. The number of movements between control and exposed groups was not different. Besides, prey detecting abilities of catsharks were not influenced by the pulse as demonstrated in Chapter 6. Also exposure to the **flatfish pulse** did not affect their prey-detecting abilities. Earlier experiments indicated that catsharks started feeding normally directly after exposure and produced eggs after 9 months (de Haan et al., 2009).

In general fright, electrotaxis or electronarcosis are three basic reactions evoked by increasing field intensity. These correspond to different phases of epilepsy suggested by Sharber and Black (1999). We found that **behavioural responses during exposure** are variable and species dependent. Roundfish species, cod in particular, were displaying more active and agitated fast swimming activity during exposure. The majority of flatfish showed only minor reactions, such as small twitching muscle contractions, and remained close to the bottom throughout the observation period. However, 15% of the exposed sole actively swam upwards during exposure. This number is lower than the 25% of active swimming sole observed in Polet et al. (2005a). Flight reactions to frequencies up to 20Hz were previously reported for sole (Stewart, 1973). When increasing the frequency as applied in the **flatfish pulse**, a cramp response and u-shaped bending are invoked in sole (Stewart, 1977; Soetaert et al., 2016a). Also in cod a cramp response was noted when applying a frequency of minimum 40Hz or a field strength of 37V/m and higher (de Haan et al., 2008; 2011; 2016). When voltage was doubled to 120V, epileptiform seizures were observed in approximately 50% of cod (Soetaert et al., 2016a,c).

Cramp reactions were also previously described for plaice, lemon sole, seabass and dogfish (D'agaro and Stravisi, 2009; Stewart 1977; de Haan et al., 2009).

Macroscopic injuries such as suffusion and multifocal petechial haemorrhages were not significantly different between exposed (**shrimp pulse**) and control groups. The electric field used to catch shrimp seemed to result in limited immediate injuries in adult fish (Chapter 3). Twenty-four hours post exposure, a small **microscopic** haemorrhage between muscle fibers was found in two exposed plaice. Additionally, in the spleen of exposed cod an increased amount of melanomacrophage centers was encountered one day after exposure. However, this was not observed in cod 14 days after being subjected to electric pulses (Soetaert et al., 2016a). No other significant histological abnormalities were observed in the liver, spleen, heart, gills, intestine or dorsal muscle, from sole, plaice, cod, bull-rout, armed bullhead, thornback ray or catshark 24h post exposure. No abnormalities were noted upon inspecting radiographs. The latter is in contrast with the **flatfish pulse** where the majority of injuries were situated in and around the vertebral column as observed in gadoids. Indeed, in 7-11% of cod and 2% of whiting caught in the field, haemorrhages opposite the vertebrae were found (Van Marlen et al., 2014). In laboratory experiments, 0-70% of animals exposed near the electrodes suffered from spinal injury or internal bleedings (de Haan et al., 2008; 2016; Soetaert et al., 2016a,b). Yet no decisive morphological or physiological parameter could explain this highly variable sensitivity to electrical induced spinal injuries. In contrast, small juvenile cod (12-16cm) was not affected by the high frequency pulse when exposed near the electrodes (de Haan et al., 2011). Also seabass (Soetaert et al., 2015), sole (Soetaert et al., 2016a), dab (de Haan et al., 2015) and dogfish (de Haan et al., 2009) did not suffer from mortality nor injuries inflicted by the flatfish pulse when observed until 5-14 days after exposure.

In Chapter 4 and 5 the effect of pulsed direct current used to catch brown shrimp on **young life stages** of cod and sole, respectively, was investigated. No significant differences in embryo mortality rate of **cod** were found. However, in the embryonic stage exposed at 18 days post fertilization (DPF), the initial hatching/developmental rate was lower. Larvae exposed at 2 and 26 days post hatching (DPH), exhibited a slightly higher mortality rate than the corresponding non-exposed groups. This seemingly negative impact in exposed cod larvae was absent in sole larvae exposed around the same developmental stage at 11 DPH. Also exposure of **sole** embryos at 2 DPF did not result in a lower survival eight days post exposure. Morphometric analysis of larvae and/or juveniles of cod and sole revealed no differences in measurements or deformations of the yolk, notochord, eye and head.

7.2.2. A critical assessment and putting into perspective of the performed studies

In what follows, various contemplations are listed related to trying and pinpoint the actual significance of our encountered results. These are to be regarded as stray thoughts that came in our minds during the various discussions on our noted findings in the framework of impact assessment of electrotrawling.

A crucial mindsetting is to realize that **every fishing technique will have an impact**. One therefore has to see the above studies in the right perspective and compare any observed effects with those of the traditionally used mechanical stimulation and not exclusively equate to unexposed control groups. Whether and to what extent fishing with traditional gear at sea causes injury was not assessed in our study. One season monitoring onboard a modified pulse trawl for brown shrimp equipped with 11 bobbins and a sieve net revealed that bycatch of plaice was significantly reduced ($p<0.001$), with 34.6%, 62.91% and 69% in June, September and October 2013 respectively (Verschueren et al., 2014). These major benefits in terms of discard reduction need to be weighed up with a critical eye against possible problems associated with pulse fishing. For example, the microscopic injury found in the lab in two out of 30, 6.7%, exposed plaice. Indeed, when assessing the impact of pulse trawling we need to balance multiple factors. The worst injuries of pulse trawling are seen on board flatfish pulse trawlers where 10% of cod suffer from spinal injury and internal bleedings. This phenomenon is mainly seen in larger individuals that are landed. Meanwhile the average catch of cod is lower in pulse trawls in comparison with traditional beam gear (van Marlen et al., 2014).

Furthermore, it is important to keep in mind that pulse trawling for brown shrimp and flatfish employ a different set-up amongst others with respect to frequency, 5Hz in comparison with more than 60Hz, respectively. Therefore, it seems evident that they may elicit varying effects on organisms in terms of behaviour, inflicted injury, fright or cramp response. To exemplify this, the spinal injury in cod is absent when trawling for brown shrimp. As such any comparison with one another should be made with caution and **both pulse trawls have to be treated differently** when arranging management, legislation and monitoring plans.

Statistically significant does not necessarily mean biologically relevant. There is no ready-made answer to the question whether the significantly higher mortality rate in exposed cod larvae, absent in sole larvae around the same developmental stage, might have negligible or marked effects or even be problematic for its recruitment at sea. Indeed, a more complex picture needs to be drawn amongst others due to the highly variable and dynamic character of the marine environment. Various issues that may have an impact on the value one is attributing to the noted negative impact in exposed cod larvae, are listed hereafter.

- The North Sea cod stock already displays a reduced reproductive capacity and might be less resilient to environmental changes. Since 1998, recruitment remains poor although there are indications of a strong 2016 year class (ICES WGNSSK, 2017). Extra pressure and additional mortality in any stage is therefore difficult to justify.
- Recruitment success greatly relies on environmental conditions such as sea surface temperature and zooplankton prey quality and quantity (Nicolas et al., 2014). Mass spawning fishes such as cod have many offspring with low survival probability in nature. For such marine teleosts, mortality exceeds 99% during the egg and larval stages (Houde, 2009). Mortality in the larval stage is mainly caused by difficulties in successful first feeding once the yolk sac is

absorbed (Stiasny et al., 2016). This is the same developmental stage at which a reduced survival in exposed larvae was noted in our studies.

- Chances that young life stages come in contact with the pulse trawling equipment are rather small. In case there would be a relevant effect of pulse trawling, one may speculate whether this has an impact on the more vulnerable, weaker individuals of a population, likely to die anyway, or are all animals affected to the same extent?
- A possible significant reduction of 32% cod bycatch as observed in September 2013 on board an electrotrawl for brown shrimp, equipped with a reduced amount of 11 bobbins and a sieve net, is a beneficial effect.

One should always remember the differences between **laboratory experiments and field studies**. It is important to be aware of the sometimes highly variable and dynamic character of the marine environment in which various parameters may change quickly at a specific site within a short time period. Studies in the laboratory have the advantage of engendering more standardized conditions. These latter may turn out to be disadvantageous when one tries to extrapolate the laboratory findings to the actual field situation. To mitigate this to some extent, in our laboratory studies, a **worst-case scenario** was applied. The included organisms were exposed for 5 seconds taking into account a trawl speed of approximately 3 knots when electrodes of 1.5m pass by. This is more than double the time a statuary animal would be in the electric field. In Chapter 6 animals were orientated perpendicularly to the electrodes to experience the highest head to tail voltage over their body. Also in the homogeneous field approach (Chapters 4 and 5), to expose young life stages, 150V/m was chosen, comparable to the high field strengths encountered in the area less than 5 cm around an electrode. Chances that small young life stages encounter such high field strengths are rather low.

Also, the electric field applied in the laboratory is not exactly the same as the one adopted in the field. Because young life stages are small, a homogeneous or uniform electric field was chosen. This field was applied in a small box, max 24*30*15cm, making it easier to expose and collect the miniscule individuals. In this way all organisms experienced the same field strength. These simplified and controlled conditions that exist in uniform **homogeneous electric fields** may be beneficial in terms of standardization as field intensity can be precisely controlled. This facilitates determination of cause and effect, but because of their more artificial nature it may be difficult to extrapolate such experimental results to real operational conditions (Snyder, 2003; Polet, 2010) and one should adopt utmost caution upon doing this. Soetaert et al. (2016a, b) illustrated that exposing animals between plate shaped electrodes had a higher impact on the animal, as a lower threshold for occurrence of epileptiform seizures in cod was observed.

Even the static non-uniform **heterogeneous** approach in the lab is only a simplification of the mobile towed fishing gear operating in the field. Also the dimensions of the water volume, the isolated tank, temperature, electrode set-up and the presence/absence of substrate are only limited examples of all the factors that may and will influence field characteristics. A moving electric field could give more information on possible escape behaviour. Effects not visible in laboratory experiments might appear in the dynamic environment at sea where multiple complex interactions with other human stressors and natural impacts appear. Housing in captivity might result in sub/super optimal conditions regarding water quality, food availability, absence of predators, … . On the other hand more realistic experiments at sea might give less reliable or should we say more difficult to interpret or putting into perspective results as a myriad of elements may contribute to the outcome. Both types of experiments are therefore necessary to complement one other.

When explaining the impacts of electric fields, some researchers suggest to not only focus on field intensity (V/m) but take into account the power transferred through a fish body, known as "**The power transfer theory**" (Kolz, 1989). Indeed, power transfer would theoretically be the best way to explain the impact on organisms. However, to standardize the amount of power transferred to the fish one need to know its conductivity, also varying with size, temperature, species, … . This is very complicated to measure and consequently also difficult to standardize. Particular problems occur when applying this concept to PDC waveforms making it not unanimously accepted by fishery researchers (Beaumont et al., 2000; 2002).

7.2.3. So where do we go from here: some recommendations for future research

Short term survival experiments with a follow-up for 24h did not reveal major impact in terms of increased mortality or morbidity. Other sub-lethal effects such as behavioural alterations (Chapter 3) and decreased prey detection ability (Chapter 6), growth impairment (Chapter 4 and 5) and lesions as perceived by RX, macroscopic and microscopic examination (Chapter 3 to 6) were not noted shortly after exposure. However, these concern only **a set of parameters** often assessed on limited time points and may not reflect the complete physiological condition of a fish guaranteeing long-term survival and reproduction (Dhert et al., 1992; Loque et al., 2000; Lund 2007). As for fish, swimming is an essential characteristic that is immediately linked to development survival and successful reproduction. In this respect, the level of swimming exercise using raceways might be used to select weak or abnormal fish and therefore more fully grasp the impact of electric pulses. Also other challenge tests or salinity stress tests (70ppt) on young life stages might reveal indirect effects (Dhert et al., 1992; Kjorsvik et al., 2003).

One promising approach that has revealed strong correlations with an organism's vitality and its probability of surviving fish capture is the Research Action Mortality Predictor (**RAMP**). This method is based on the innate capability to respond to reflex stimuli. Pilot experiments whereby 30 soles were exposed to the flatfish pulse did not reveal differences with the control group in terms of responses to reflex stimuli (data not shown). Electric pulses applied together with oxygen depletion resulted in statistically significant differences between exposed and control fish, but it was not possible to link the observed differences to the interaction with oxygen deficit and pulse or the mere oxygen depletion. Earlier field experiments indeed revealed oxygen depletion as an important parameter to influence the RAMP score (Uhlman et al., 2016). According to van der Reijden et al. (2017), fish were more vital and reflexes less impaired in the flatfish pulse trawl compared to traditional tickler chain beam trawl.

Nevertheless, parameters such as RAMP and type and extent of injury in fish are typically determined by external visual examination relying on observers. **Gross macroscopic examination** to describe semi-quantitative indices may be prone to observer subjectivity and more disadvantageous in terms of quantitative analytical capacity. This possible bias in scoring injuries may be eliminated by taking standardized, high resolution colour images. Especially the coverage of injuries such as visible multifocal cutaneous petechiae, point bleedings, and suffusion or haemorrhaging, bruising, may be more accurately recorded. The injury surface area relative to the whole fish/fillet may be automatically computed. The use of forensic techniques, such as fluorescein, may enhance the detection and quantification of latent epithelial damage in fish not visible to the naked eye (Colotelo et al., 2009; 2011) rendering this technique more sensitive for assessing possibly inflicted lesions. Fluorescein is a compound that adheres to wounds and areas of mucus and scale loss and may be visualized as green fluorescence when illuminated with ultraviolet light (Noga and Udomkusonsri, 2002). This way of examining the fish may rectify or disprove the hypothesis that the electric pulses render the fish more susceptible to skin lesion development by inducing an altered skin morphology leading to a breach in the barrier function. Indeed, the resident skin microbiota in fish may harbour organisms that can invade damaged skin.

The studies hitherto carried out only were **short term** with observation periods of 24 hours for adults and maximum 3 weeks for young life stages. Any conclusions on the long-term impact of electric pulses therefore may be assigned as speculative warranting further research with functioning of the immune and reproductive system, chronic stress levels, growth and possible deformations as possible parameters.

Rearing **young life stages** is often accompanied by high and unpredictable mortality. This may not only be related to generally higher mortality rates in earlier stages but also to spawning stock quality. Additionally multiple abiotic and biotic factors may impact embryo and larval survival rendering standardization of and limiting variation in survival rate between tanks a major challenge. Because of the high variation in survival encountered in between tanks when culturing Atlantic cod (Chapter 4), the amount of replicates was increased from 3 to 7-10 for the sole experiment (Chapter 5). In similar impact studies using sole, even 15 replicates were used (Bolle et al., 2012). This was only feasible when using smaller tanks with a reduced number of individuals per replica. Instead of using 25L cylindro-conic flow through tanks used in the cod experiment, small 1L beakers with 20-30 individuals were installed. A disadvantage of the small beakers was that even with water replacement every second day, it was difficult to remove dead eggs or larvae and consequently bacterial and fungal load. Dead organisms may deteriorate the water quality and hence affect the survival of other embryos or larvae. Therefore, it might be worth considering culturing larvae individually. Sole larvae may indeed be cultured in 24 well-plates serving as larval culture chambers (De Swaef et al., accepted). This ensures a standardised and reliable experimental model in which the possible death of one larva has no impact on the other larvae. Implementing a well plate system also facilitates monitoring of the health status and behaviour of the individually housed larvae. These parameters, in addition to mortality, may be adopted to assess the impact of electric pulses. Consequently, the number of replicates and statistical power of the experiment would increase and meanwhile the amount of experimental animals needed reduced.

The precautionary approach is still warranted when making statements on the impact of electric pulses on young marine organisms. In our studies including sole only two life stages were investigated, that is at 2DPF and 11DPH. However, larvae may be more at risk to get in contact with the electric field when settling at the seafloor about 3 weeks after hatching (Fonds, 1979; van der Veer et al., 2001). This transition to a benthic life, is typically associated with very complex anatomical transformations such as the migration of the eye and rotation in body position before attaining a juvenile form (Palazzi et al., 2006; Piccinetti et al., 2012). These complex morphological and physiological changes in terms of pigmentation and metamorphosis render the larvae very sensitive to stressors, warranting the need to investigate the impact of electric pulses on this life stage. Besides adopting a morphological approach when assessing this, also the expression of the genes involved in metamorphosis may be studied when sampling at for example at 24 and 36 DPH (17°C). Additionally, in sole, malpigmentation will only be visible in the juvenile stage, stressing the need to keep the larvae for a longer period following exposure.

Also cod larvae descend from the water column to bottom habitats as they become older (Yin and Blaxter, 1987; Heesen and Rijnsdorp, 1989) at sizes of 2.5-6 cm, when a complete transformation to the juvenile stage occurs (Fahay 1983; Lough et al., 1989). No cod juveniles larger than 2.4 cm were investigated in our trials. Longer observation periods and exposure of other life stages may be warranted to fully investigate the impact of electrotrawling for brown shrimp. Muth & Rupert (1997) reported that differences in growth might be not detectable until 21 days after treatment.

Hitherto, no studies on the impact of electric pulses on the **reproduction of adult brood stock** were performed nor on **fertility success of exposed gametes**. Exposure of egg carrying freshwater fish to electric fields may cause significant damage or premature expulsion of gametes and sometimes reduced viability of subsequently fertilized eggs (Muth and Ruppert 1997; Roach 1996, Roach 1999). To what extent a similar story is to be told for marine fish embryo's and larvae can only be speculated on as interspecific differences may occur. Other species, such as herring, also need to be investigated since demersal eggs are produced (Yin and Blaxter, 1987) which could be exposed when electrodes are towed over the sea bed.

So far research on the effects of electrofishing on marine organisms is limited **to single exposure events**. However certain fishing grounds may be fished intensely during particular seasonal periods (Van Denderen, 2015; Piet & Hintzen, 2012). Five percent of the North Sea surface area is trawled more than five times a year (Eigaard et al., 2014). Only Soetaert et al. (2016c) investigated the effect of 20 repetitive exposures in four days on shrimp but did not find a decreased survival. Likewise, multiple exposures with intervals of 1-5 min did not appear to cause major harm to zebra fish embryo's (Nutile et al., 2012). Again, utmost caution is warranted when extrapolating these data to other marine species.

In order to assess the impact of electrotrawling, one should in principle investigate all possible **components of and interactions within the ecosystem**. So far experiments focussed mainly on macrobenthic species, such as fish and epibenthic invertebrates like crustaceans, at high trophic levels in the top layers of the food web. Hitherto, impact on organisms at lower trophic levels involved in primary but also secondary production is not known. This information nevertheless is crucial, as, photosynthetic organisms such as phytoplankton/benthos, dominated by microalgae as diatoms and dinoflagellates, are often the fundamental food source on which marine ecosystems are based (Field et al., 1998; Falkowski et al., 2000; Kaiser et al., 2005). Electrical currents might influence chemical or physical interactions within sediments and consequently disturb inhabiting small organisms, meio zoobenthos, such as copepods being significant in nutrient cycling and carbon turn over, or foraminifera, important bioindicators for pollution and ocean acidification (Nixon, 1981; Roman et al., 1988; Walve and Larson, 1999, Calbet and Saiz 2005; Frontalini and coccioni; Alves Martins et al., 2016). To our knowledge the impact of electric pulses on bacteria has never before been investigated. In fact, bacteria might function as an electron transporter, simulating a living electric cable generating and conducting electricity across centimeter-scale distances (Pfeffer et al., 2012; Malkin and Meysman, 2015), highlighting the need for research on the impact of electric pulses on these micro-organisms.

Each study meant to solve a research question often generates more questions, rendering any research in the marine environment challenging and intricate. On the other hand, time and research money may be limited. Visions differ on when sufficient scientifically valid results are available. A new approach that started in 2015 with the **international pulse dialogue meetings**, involving different stakeholders, should bring more transparency on benefits, questions and concerns (Kraan et al., 2015; Steins et al., 2017). A multi-annual research programme started in 2016 to address some of the most important problems. Predictive models of the (**long-term**) effect of electric pulses on organisms, under various environmental conditions and on the functioning of the benthic ecosystem will be developed by means of field and laboratory experiments. The results will be integrated into a spatially predictive model of distribution and consequences of pulse fishing activities and synthesized in an Impact Assessment.

At one point science stops and policy comes in. Developing a European guideline when evaluating new approaches or future technical innovations is an avenue well worth exploring. The ultimate goal is having and maintaining healthy oceans and sustainable fisheries.

7.3. Expanding on other areas of concern that have emerged from pulse trawling for brown shrimp

Although hitherto the immediate negative effects of pulse trawling seem fairly small in comparison with traditional beam trawling, every action might give a reaction and possible effect on ecosystems where human influence is intertwined with natural dynamics (Meire et al., 2005). Ecosystem resilience can weaken considerably when environmental and human sources of stress act simultaneously (Kaiser et al., 2005). Changes in fishing activity may have top-down or bottom-up effects and trophic cascades on communities with many more aspects to elaborate upon. A short overview of often raised concerns is described below (Kraan et al., 2015).

7.3.1. Increased efficiency

Electrical fisheries for shrimp, Hovercran combined with a bobbin rope, are getting **more efficient**. Indeed, these can catch shrimp during daytime and in clear water which is in contrast with traditional gear that is mostly shrimping at night or in turbid water. This is exemplified by more market sized shrimp being caught in summer, 16% in June and 9.4% in September 2013 (Verschueren et al., 2014). Also a catch increase of 30.8% was observed during September-October 2016 (Verschueren et al., 2016). Spatial differences exist, for instance in German waters, the increase in catch efficiency is less explicit due to a general increase in turbidity of the sea water (Kratzer et al., 2012). Besides, catch efficiency of a fishing gear may increase over time due to technological developments and improved skills of the fishermen, in particular when new techniques are introduced (Eigaard et al., 2014). Increased efficiency could be an advantage in terms of bottom disturbance per kg of shrimp caught and less bycatch or discards, 50-76% fish and benthos and 19-33% undersized shrimp (Verschueren et al., 2014). However, it was suggested that negative feedback processes due to reductions of trawling disturbance might result in a reduction of benthos and consequently of the fish that eat them. Indeed, some fishermen state that the seabed must be ploughed for the aforementioned reason (Verweij and van Densen, 2010; Beare et al., 2013).

On the other hand, increased efficiency should have considerations on stock effect and management consequences. Currently the shrimp fishery is largely unregulated. Only the number of fishing licenses and some technical measures (mesh, sieve net and engine size) are being controlled. Regulations limiting the fishing effort or the annual Total Allowable Catch (TAC) or quota are not existing. The fishery has long been considered sustainable (Welleman and Daan, 2001). Also, the overall landed catches of Brown Shrimp remained at a high level indicating that the stock was not declining (ICES, 2000; Polet et al., 2004). The highest recorded landings of 38 000 and 37 500 t were in 2005 and 2014, respectively (ICES, 2015). However, landings of *C. crangon* consistently increased since the 1970s probably due to a decrease in predation, by cod and whiting, and an increase in fishing effort and efficiency (Temming and Hufnagl, 2015). Although the number of active vessels has been constant, engine and unmonitored deck equipment power continuously increased, resulting in a technological creep. Nowadays there are strong indications that high fishing pressure likely led to growth **overfishing of the shrimp population** (ICES, 2015; Tulp et al., 2016). Shrimp are harvested at a suboptimal average size and prices recently reached a record high with an increase of 40% in two years (ICES 2014a; ICES, 2016).

The effect that unregulated exploitation accompanied with more efficient electro trawling devices can have on shrimp stocks is illustrated by the drastic decline in shrimp biomass in the **East Chinese Sea**

(ECS) around the 1990's caused by misuse of shrimp electrical pulse stimulus apparatus (SEPSA) (Yu et al., 2007). Excessive power output and improper settings of pulse parameters resulted in poor selectivity and high mortality of juvenile shrimp. Also, the large-scale and illegal use of SEPSA together with an unregulated fishery contributed to the decline. In 2001 this technique was banned from the waters off Zhejiang Province and other parts of the ECS.

Nevertheless, technological progress resulting in more efficiency does not need to end in a downward spiral. Potential steps towards a Brown Shrimp **management** were developed (ICES, 2013b; ICES, 2015). Unfortunately, due to the short life span of *C. crangon* an annual stock assessment and annual TAC rule are not suitable. Therefore, the fishing industry has proposed a landing per unit effort (lpue) based harvest control rule, in order to obtain an MSC label (ICES, 2014a). Additionally, the commercial pulse equipment offers only little variability of electrical settings. Only the pulse amplitude is adjustable on a scale from 0-100% with in general the best results at 80% output. This enables to cope with variations in conductivity varying with temperature/season and salinity. However, Marelec nv. has increased the amplitude up to 100V. Besides, one always assumed that increasing the pulse amplitude and consequently the electric field strength above 80% of the generator output, results in lower catch volumes of shrimp. Unfortunately, recent research on the O 82 vessel revealed that this might have been rather an exception. Increasing the amplitude does mean increasing catches and bycatch at least in absolute numbers (pers. Comm. Maarten Soetaert). Additionally, the use of bobbins together with a pulse system is questioned. Also the discussion of allowing pulse trawl systems in natura 2000 areas is ongoing.

Available evidence showed that also flatfish pulse trawling seems to have a higher catch efficiency for the target species sole but lower for plaice and other fish species. Control and enforcement procedures and certification of the system are being developed for flatfish fishery.

7.3.3. Economic, social and cultural implications

Management, stock- and ecosystem-based indicators are used to assess the fishing impact and good environmental status of European seas (EU, 2008). The common fisheries policy goes further and additionally includes economic, social and cultural concerns. Regarding socio economics, electrotrawling entails more catch and less work load for fishermen. Increased efficiency, however, may hold risks and lead to increased fishing capacity of the fleet. Without the right management, control and enforcement tools, this might lead to overfishing or to a further reduction of the fishing fleet potentially resulting in bankruptcy and unemployment. Clear rules are therefore needed regarding design and efficiency of pulse trawls. The combined use of pulse trawls with bobbins is much debated. Besides, the inherent higher catching efficiency of the pulse trawl for the target species, recent research demonstrated that increasing pulse amplitude may lead to an additional higher catch efficiency of brown shrimp. Fixed certification systems or monitoring with black box systems should prevent misuse of increasing pulse parameters. On the other hand, some flexibility is essential to allow for improvement and innovation.

Purchasing and maintaining the technology on board is expensive. The price of a complete system including two beams, pulse generator, cables etc. is currently estimated at about 70,000 Euro (ICES, 2012). Fishermen who cannot invest in a pulse trawl will fish behind the net and earn less than their pulse trawling colleagues. Especially when the different fleets operate on the same fishing grounds. It is known when new technologies are introduced, fish is more difficult to catch (Arlinghaus et al., 2017), in particular for the less modern vessels. On the other hand, if the technique is restricted to a delimited area, as is the case for pulse trawling in the southern North Sea, this may engender the perception of an **unfair competition** with fishermen fishing outside this zone. Besides, upscaling of the fishery to approximately 80 pulse vessels has damaged trust.

Ethical concerns about animal welfare are often raised. Some argue that the use of electrical pulses is cruel. What fish feel is much debated (Chanderoo et al., 2004; Ashley et al., 2007; Rose et al. 2002;

Braithwaite 2010; Rose et al., 2014). In the face of this doubt, welfare precautions of fish require consideration.

The combination of electricity and water is regarded as potentially dangerous and therefore electrofishing may be perceived negatively by the general public possibly leading to terms as “electrocution” being used. Therefore, scientific research should not only be reported in peer-reviewed journals but also be provided to non-scientific stakeholders whereby these latter have access to objective and clear information.

Take home message:

Although the direct short-term impact of electro trawling for brown shrimp hitherto seems limited, long term effects and various components of the marine ecosystem have not yet been investigated. As such, major gaps in knowledge still exist warranting further research on this alternative fishing technique to be able to safeguard our oceans and ensure sustainable fisheries.

Nothing lasts, nothing is finished and nothing is perfect

References

- Abarnou, A. and Miossec, L. 1992. Chlorinated waters discharged to the marine environment chemistry and environmental impact. An overview. The Science of the Total Environment, 126: 173-197.
- Agius, C.; Roberts, R. 2003. Melano-macrophage centres and their role in fish pathology. Journal of Fish Diseases. 26:499-509.
- Alvarez-Iglesias, P. and Rubio, B. 2009. Redox status and heavy metal risk in intertidal sediments in NW Spain as inferred from the degrees of pyritization of iron and trace elements. Marine Pollution Bulletin 58, 542–551.
- Alves Martins, M.V., Souza Pinto, A.F., Frontalini, F., Machado da Fonseca, M.C., Terroso, D.L., Laut, L.L.M., Zaaboub, N., da Conceição Rodrigues, M.A., Rocha, F. Can benthic foraminifera be used as bio-indicators of pollution in areas with a wide range of physicochemical variability? Estuarine, Coastal and Shelf Science 182, Part A 211-225,
- Andersen, O. G. N., Nielsen, J. R., and Smidt, E. L. B. 1994. Description and comparison of eggs and yolk sac larvae of Greenland cod (*Gadus ogac*), Atlantic cod (*Gadus morhua*) and Arctic cod (*Boreogadus saida*) from West Greenland waters. Journal of Northwest Atlantic Fishery Science, 16: 19-32.
- Anderson, W.G., 2012. The endocrinology of 1α-hydroxycorticosterone in elasmobranch fish: A review. Comparative Biochemistry and Physiology, Part A 162:73-80.
- Arlinghaus, R., Laskowski, K.L., Alós, J., Klefoth, T., Monk, C.T., Nakayama, S., Schröder, A., 2017. Passive gear-induced timidity syndrome in wild fish populations and its potential ecological and managerial implications. Fish and Fisheries 2017.18 360-373
- Arnott, S., Neil, D., Ansell, A., 1998. Tail-flip mechanism and size dependent kinematics of escape swimming in the brown shrimp Crangon crangon. J. Exp. Biol. 201, 1771–1784.
- Ashley P.J., Sneddon L.U., McCrohan C.R. 2007. Nociception in fish: stimulus-response properties of receptors on the head of trout *Oncorhynchus mykiss*. Brain Res. 2007 Aug 29;1166:47-54. Epub 2007 Jul 17.
- Auditore, P. J., Lough, R. G., and Broughton, E. A. 1994. A review of the comparative development of Atlantic cod (*Gadus morhua* L.) and Haddock (*Melanogrammus aeglefinus* L.) based on an illustrated

series of larvae and juveniles from the Georges Bank. Northwest Atlantic Fisheries Organization Scientific Council Studies, 20: 7-18.

- Arimoto, M., Sato, J., Maruyama, K., Mimura, G. and Furusawa, I. 1996. Effect of chemical and physical treatments on the inactivation of striped jak nervous necrosis virus (SJNNV). Aquaculture 143:15-22.
- Barton BA (2002). Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integr Comp Biol 42: 517-525
- Baum, J. K. and Worm, B. 2009. Cascading top-down effects of changing oceanic predator abundances. Journal of Animal Ecology 78, 699–714.
- Baynes, S.M., Howell, B.R., Beard, T.W. 1993. A review of egg production by captive sole, *Solea solea* (L.) Aquaculture Research, 24:2.
- Beare, D., Rijnsdorp, A.D., Blaesberg, M., Damm U., Egekvist J., Fock, H., Kloppmann, M., Röckmann, C., Schroeder, A., Schulze, T., Tulp, I., Ulrich, C., van Hal R., van Kooten, T., Verweij, M., 2013. the effect of fishery closures: Lessons learnt from the Plaice Box. Journal of Sea Research 84 (2013) 49–60
- Beaumont, W.R.C., Taylor, A.A.L., Lee, M.J. and Welton, J.S. 2002. Guidelines for Electric Fishing Best Practice. Environment Agency R&D Technical Report W2-054/TR. pp206.
- Beaumont, W.R.C., Peirson, G. and Lee, M.J. 2006. Factors affecting the characteristics and propagation of voltage gradient fields from electric fishing anodes. Fisheries Management and Ecology; 13; 47-52.
- Bedore, C.N., and Kajiura, S.M. 2013. Bioelectric fields of marine organism: voltage and frequency contributions to detectability by electroreceptive predators. Physiological and Biochemical Zoology 86(3), 298-311.
- Berghahn, R., and Purps, M. 1998. Impact of discard mortality in *Crangon* fisheries on year-class strength of North Sea flatfish species. Journal of Sea Research, 40: 83-91.
- Berghahn, R., 1996. Episodic Mass Invasions of Juvenile Gadoids into the Wadden Sea and their Consequences for the Population Dynamics of Brown Shrimp (Crangon crangon). Marine Ecology, 17:1-3
- Berghahn, R., Waltemath, M., and Rijnsdorp, A. D. 1992. Mortality of fish from the by-catch of shrimp vessels in the North Sea. Journal of Applied Ichthyology, 8: 293–306.
- Bergman, M.J.N. & Hup, M. 1992. Direct effects of beamtrawling on macrofauna in a sandy sediment in the southern North Sea. ICES J. Mar. Sci. 49, 5-11.

- Bohl, R. J., Henry, T. B., and Strange, R. J. 2010. Electroshock-induced mortality in freshwater fish embryos increases with embryo diameter: a model based on results from 10 species. Journal of Fish Biology, 76: 975–986.
- Bolle, L.J., de Jong, C.A.F., Bierman, S.M., van Beek, P.J.G., van Keeken, O.A., Wessels, P.W., van Damme C.J.G., Winter H.V., de Haan D., Dekeling, R.P.A. 2012. Common Sole Larvae Survive High Levels of Pile-Driving Sound in Controlled Exposure Experiments. PLoS ONE 7(3): e33052. doi:10.1371/journal.pone.0033052
- Boonstra, G.P. and de Groot, S.J. 1974. The development of an electrified shrimp-trawl in the Netherlands. Journal du Conseil/Conseil International pour l'Exploration de la Mer 35(2), 165–170.
- Borucinska, J., and Frasca, S. 2002. Naturally occurring lesions and micro-organisms in two species of free-living sharks: the spiny dogfish, *Squalus acanthias* L., and the smooth dogfish, *Mustelus canis* (Mitchill), from the north-western Atlantic. Journal of fish diseases, 25: 287-298.
- Braithwaite V. 2010. Do Fish Feel Pain? Oxford University Press: 208pp. ISBN 0191613967, 9780191613968
- Breen, M., Howell, T., and Copland, P. 2011. A report on electrical fishing for razor clams (*Ensis. Sp.*) and its likely effects on the marine environment. Marine Scotland Science Report 03/11
- Brown, J. A., Minkoff, G., Puvanendran, V., 2003. Larviculture of Atlantic cod (*Gadus morhua*): Progress, protocols and problems. Aquaculture, 227: 357-372.
- Calbet, A., Saiz, E. 2005. The ciliate-copepod link in marine ecosystems. Aquatic Microbial Ecology 38: 157–167
- Campos, J., and Van der Veer, H. W. 2008. Autecology of *Crangon crangon* (L.) with an emphasis on latitudinal trends. Oceanography and Marine Biology: An Annual Review, Vol 46, 46: 65-104.
- Campos J., 2009. The eco-geography of the brown shrimp Crangon crangon in Europe. ISBN 978-90-865-9350-7
- Catchpole, T. L., Revill, A. S., Innes, J., and Pascoe, S. 2008. Evaluating the efficacy of technical measures: a case study of selection device legislation in the UK Crangon crangon (brown shrimp) fishery. ICES Journal of Marine Science/ Journal du Conseil, 65: 267-275.
- Chandroo KP, Duncan IJH, Moccia RD 2004. Can fish suffer? Perspectives on sentience, pain, fear and stress. Appl Anim Behav Sci 86:225–250

- Chen, C., Smye, S. W., Robinson, M. P., and Evans, J. A. 2006. Membrane electroporation theories: a review. Medical and Biological Engineering and Computing, 44: 5–14.
- Cho, G. K., Heath, J. W., and Heath, D. D. (2002). Electroshocking influences chinook salmon egg survival and juvenile physiology and immunology. Transactions of the American Fisheries Society, 131: 224–233.
- Colotelo, A.H., Cooke, S.J., Smokorowski, K.E., 2009. Application of forensic techniques to enhance fish conservation and management: injury detection using presumptive tests for blood. Endang Species Res Vol. 9: 169–178 doi: 10.3354/esr00178
- Colotelo, A.H., Cooke, S.J., 2011. Evaluation of common angling-induced sources of epithelial damage for popular freshwater sport fish using fluorescein. Fisheries Research 109 (2011) 217–224
- Conway, D. V. P., Coombs, S. H. and Smith, C. 1997. Vertical distribution of fish eggs and larvae in the Irish Sea and southern North Sea. ICES Journal of Marine Science, 54: 136–147.
- D'Agaro, E. and Stravisi, A. 2009. Numerical simulation of electrofishing in seawater. Italian Journal of Animal Science, 8: 633-645.
- Dalbey, S. R., McMahon, T. E. and Fredeberg, W. 1996. Effect of Electrofishing Pulse Shape and Electrofishing-Induced Spinal Injury on Long-Term Growth and Survival of Wild Rainbow Trout. North American Journal of Fisheries Management, 16: 560-569.
- De Groot, S.J. and Boonstra, G.P. 1970. Report on the development of an electrified shrimp-trawl in The Netherlands. ICES CM. 1970/B:5.
- de Haan D., Fosseidengen J.E., Fjelldal P.G., Burggraaf D., Rijnsdorp A.D. 2016. Pulse trawl fishing: characteristics of the electrical stimulation and the effect on behaviour and injuries of Atlantic cod (*Gadus morhua*). ICES Journal of Marine Science 73: 1557-1569.
- de Haan, D. de, Haenen, O., Chen, C., Hofman, A., Es, Y. van, Burggraaf, D. and Blom, E. 2015. Pulse trawl fishing: The effects on dab (*Limanda limanda L.*). IMARES Report number C171/14.
- de Haan, D., Fosseidengen, J.E., Fjelldal, P.G. and Burggraaf, D. 2011. The effect of electric pulse stimulation to juvenile cod and cod of commercial landing size, IMARES Report number C/141/11: 44 pp.
- de Haan, D., van Marlen, B., Velzenboer, I., van der Heul, J. and van der Vis, H. 2009. The effect of pulse stimulation on biota - Research in relation to ICES advice - Effects on dogfish. ICES Document C105/09. 32 pp.

- de Haan, D., van Marlen, B., Kristiansen, T.S. and Fosseidengen, J.E. 2008. The effect ofpulse stimulation on biota – Research in relation to ICES advice – Progress Report on the Effects to Cod. IMARES Report C98/08, 22 pp.
- Dijkgraaf, S. and Kalmijn, A. J. 1966. Versuche Zur Biologischen Bedeutung Der Lorenzinischen Ampullen Bei Den Elasmobranchiern. Zeitschrift Fur Vergleichende Physiologie, 53: 187-.
- Depestele, J., Desender, M., Benoît, H. P., Polet, H. and Vincx M. 2014. Short-term survival of discarded target fish and non-target invertebrate species in the "eurocutter" beam trawl fishery of the southern North Sea. Fisheries research, 154: 82-92.
- Depestele, J., Ivanović, A., Degrendele, K., Esmaeili, M., Polet, H., Roche, M., Summerbell, K., Teal, L.R., Vanelslander, B. and O'Neill F.G. 2015. Measuring and assessing the physical impact of beam trawling. ICES Journal of Marine Science: doi:10.1093/icesjms/fsv056
- Desender, M., Chiers, K., Polet, H., Verschueren, B., Saunders, J., H., Ampe, B., Mortensen, A., Puvanendran, V., and Decostere, A., 2016. Short-term effect of pulsed direct current on various species of adult fish and its implication in pulse trawling for brown shrimp in the North Sea. Fisheries Research, 179: 90-97.
- Desender, M., Kajiura, S., Ampe, B., Dumolein, L., Polet, H., Chiers, K. and Decostere A. 2017a. Pulse trawling: evaluating its impact on prey detection by small-spotted catshark (*Scyliorhinus canicula*). Journal of Experimental Marine Biology and Ecology 486; 336-343
- Desender, M., Decostere, A., Adriaens, D., Duchateau, L., Mortensen, A., Polet, H., Puvanendran, V., Verschueren, B. and Chiers, K. 2017b. Impact of pulsed direct current on embryonated eggs, larvae and young juveniles of Atlantic cod (*Gadus morhua*) and its implication in electrotrawling for brown shrimp. Marine and Coastal Fisheries 9; 330-340
- Desender, M., Dumolein, L., Duchateau, L., Adriaens, D., Delbare D., Polet, H., Chiers, K., and Decostere, A. 2018. Electrotrawling: The impact of pulsed direct current on early life stages of sole (*Solea solea*). (In press: North American Journal of Fisheries Management)
- De Swaef, E., Van den Broeck, W., Dierckens, K. and Decostere, A. 2015. Disinfection of teleost eggs: a review. Reviews in Aquaculture 7: 1–21
- De Swaef, E., Demeestere K., Boon, N., Van den Broeck, W., Haesebrouck, F., Decostere, A. 2017. Development of a Reliable Experimental Set-Up for Dover Sole Larvae *Solea Solea* L. And Exploring the Possibility of Implementing This Housing System in a Gnotobiotic Model. Res Vet Sci 115, 418-424

- Dhert, P., Lavens, P., Sorgeloos, P. 1992. Stress evaluation: A tool for quality control of hatchery-produced fish and shrimp fry. Aquaculture Europe 17: 6-10.
- Doeksen, A. 2006. Ecological perspectives of the North Sea *C. crangon* fishery: An inventory of its effects on the marine ecosystem. BSc Thesis. Wageningen University. Wageningen. 133 pp.
- Dolan, C. R. and Miranda, L. E. 2003. Immobilization thresholds of electrofishing relative to fish size. In Transactions of the American Fisheries Society, pp. 969-976.
- Dwyer, W. P., Fredenburg, W. A., and Erdahl, D. A. 1993. Influence of electroshock and mechanical shock on survival of trout eggs. North American Journal of Fisheries Management, 13: 839–843.
- Dwyer, W. P., and Erdahl, D. A. 1995. Effects of electroshock voltage, wave form, and pulse rate on survival of cutthroat trout eggs. North American Journal of Fisheries Management, 15: 647–650.
- Eigaard, O. R., Marchal, P., Gislason, H., and Rijnsdorp, A. D. 2014. Technological Development and Fisheries Management. Reviews in Fisheries Science & Aquaculture, 22: 156-174.
- Emery, L. 1984. The physiological effects of electrofishing. In California-Nevada Wildlife Transactions, pp. 59-62.
- EU, 1998. Council Regulation (EC) No. 850/98 of 30 March 1998 for the conservation of fishery resources through technical measures for the protection of juveniles of marine organisms (OJL 125, 27.4.1998), 55pp.
- EU, 2008. EC. Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). Off J Eur Union 2008:19–40.
- EU, 2009. Council Regulation (EC) No. 43/2009 of 16 January 2009 fixing for 2009 the fishing opportunities and associated conditions for certain fish stocks and groups of fish stocks, applicable in Community waters and, for Community vessels, in water where catch limitations are required. (OJ L22, 26.1.2009), 205pp.
- EU, 2013. Art 14 of the Eu Regulation No 1380/2013 of the European Parliament and of the Council of 11 December 2013 on the Common Fisheries Policy, amending Council Regulations (EC) No 1954/2003 and (EC) No 1224/2009 and repealing Council Regulations (EC) No 2371/2002 and (EC) No 639/2004 and Council Decision 2004/585/EC.

- Fahay, M.P. 1983. Guide to the early stages of marine fishes occurring in the western North Atlantic Ocean, Cape Hatteras to the southern Scotian Shelf. Journal of Northwest Atlantic Fishery Science, 4: 1-423.
- FAO, 2009. Fisheries management. The ecosystem approach to fisheries. FAO Technical Guidelines for Responsible Fisheries, 4 (Suppl. 2). 112 pp. ISBN 978-92-5-106424-5
- FAO, 2011. Code of conduct for responsible fisheries. FAO publication 108pp. ISBN 978-92-5-106708-6
- FAO, 2012. State of world fisheries and aquaculture. 230pp. ISBN 978-92-5-107225-7
- Ferguson, H., Bjerkas, E., Evensen, O., 2013. Systemic Pathology of Fish: A Text and Atlas of Normal Tissue Responses in Teleosts, and Their Responses in Disease. Scotian press. 368 pp.
- Filer, J. L., Booker, C. G. and Sims, D. W. 2008. Effects of environment on electric field detection by small spotted catshark Scyliorhinus canicula (L.). J Fish Biol, 72: 1450-1462.
- Fonds M 1979. Laboratory observations on the influence of temperature and salinity on development of the eggs and growth of the larvae of Solea solea (Pisces). Mar Ecol Prog Ser 1: 91–99.
- Fonds, M.R., Cronie, R., Vethaak, A.D., Van Der Puyl, P. 1992. Metabolism, food consumption and growth of plaice (Pleuronectes platessa) and flounder (Platichthys flesus) in relation to fish size and temperature. Netherlands Journal of Sea Research, 29: 1–3
- Fonteyne, R. and Polet, H. 2002. Reducing the benthos by-catch in flatfish beam trawling by means of technical modifications. Fisheries Research, 55: 219-230.
- Frontalini, F. and Coccioni, R., 2011. Benthic foraminifera as bioindicators of pollution: A review of Italian research over the last three decades. Revue de Micropaléontologie, 54:2
- Gaylor, D. C., Prakahasante, K., and Lee, R. C. 1988. Significance of cell-size and tissue structure in electrical trauma. Journal of Theoretical Biology, 133: 223–237.
- Gill, A. B., Gloyne-Philips, I., Kimber, J. A. and Sigray, P. 2014. Marine Renewable Energy, Electromagnetic (EM) fields and EM-sensitive animals. Marine Renewable Energy Technology and Environmental Interactions, Humanity and the Sea, 62-79.
- Gill, A. B. and Kimber, J. A. 2005. The potential for cooperative management of elasmobranchs and offshore renewable energy development in UK waters. Journal of the Marine Biological Association of the U.K., 85, 1075–1081.

- Gill, A. B. and Taylor, H. 2001. The potential effects of electromagnetic fields generated by cabling between offshore wind turbines upon elasmobranch fishes. Research Project for the Countryside Council for Wales, CCW Science report 488.
- Glauert, A. M. 1987. Fixation, Dehydration and Embedding of Biological Specimens, 1st edn. Amsterdam: Elsevier Science.
- Godfrey, H. 1957. Mortalities among developing trout and salmon ova following shock by direct-current electric fishing gear. Journal of the Fisheries Research Board of Canada, 14: 153–164.
- Graham, N. 2003. By-catch reduction in the brown shrimp, *Crangon crangon*, fisheries using a rigid separation Nordmøre grid (grate). Fisheries Research, 59: 393-407.
- Grotmol, S., Dahl-Paulsen, E., and Totland, G. K. 2003. Hatchability of eggs from Atlantic cod, turbot and Atlantic halibut after disinfection with ozonated seawater. Aquaculture 221: 245–254
- Hagerman L. 1970. Locomotory activity patterns of Crangon vulgaris (Fabricius) (Crustacea, Natantia).Ophelia 8, 255-266.
- Haine, O. S., Ridd, P. V. and Rowe, R. J. 2001. Range of electrosensory detection of prey by Carcharhinus melanopterus and Himantura granulata. Mar Freshw Res 52:291–296
- Hall, T. E., Smith, P., Johnston, I. A., 2004. Stages of embryonic development in the Atlantic cod *Gadus Morhua*. Journal of Morphology, 259: 255-270.
- Halsband, E. 1967. in Fishing with electricity – its applications to biology and management Contributions to a Symposium (Vibert, R., editor). Basic Principles of Electric Fishing, Fishing News (Books) Ltd, London.
- Hamre, K., 2006. Nutrition in cod (*Gadus morhua*) larvae and juveniles. Ices Journal of Marine Science 63: 267–274.
- Hansen, Ø. J. and Puvanendran, V. 2010. Fertilization success and blastomere morphology as predictors of egg and juvenile quality for domesticated Atlantic cod, *Gadus morhua*, broodstock
- Hansen, Ø. J., Puvanendran, V. and Bangera, R. 2015. Do maternal age and experience contribute to better growth, survival and disease resistance of offspring in Atlantic cod (Gadus morhua)? Aquaculture International 23: 1157–1164.
- Hayes, F. R. 1949. The growth, general chemistry, and temperature relations of salmonid eggs. The Quarterly Review of Biology, 24: 281–308.

- Heesen, H.J.L., and Rijnsdorp A. D. 1989. Investigation on egg production and mortality of cod (Gadus morhua L.) and plaice (Pleuronectes platessa L.) in the southern and eastern North Sea in 1987 and 1988. Rapp. P. v. Réun. Cons. int. Explor. Mer, 191: 15-20.
- Henry, T. B., and Grizzle, J. M. 2003. Electroshocking-induced injuries in newly transformed juvenile fish. Journal of Aquatic Animal Health, 15: 147–157.
- Henry, T. B., Grizzle, J. M., and Maceina, M. J. 2003. Electrofishing-induced mortality of four fish species during posthatching development. Transactions of the American Fisheries Society, 132: 299–306.
- Henry, T. B., and Grizzle, J. M. 2004. Survival of largemouth bass, bluegill and channel catfish embryos after electroshocking. Journal of Fish Biology, 64: 1206–1216.
- Henry, T. B., and Grizzle, J. M. 2006. Electric-induced mortality of newly transformed juvenile fishes in waters of different conductivity. Journal of Fish Biology, 68: 747–758.
- Herbing, I. H. V., Miyake, T., Hall, B. K., and Boutilier, R. G. 1996. Ontogeny of feeding and respiration in larval Atlantic cod *Gadus morhua* (Teleostei, Gadiformes): I. Morphology. Journal of Morphology, 227: 15-35.
- Houde E.D. 2009. Chapter 3: Mortality. Fishery science: The unique contributions of early life stages. edited by Lee A. Fuiman, Robert G. Werner. John Wiley & Sons, Technology & Engineering - 336 pages.
- ICES, 2000c. Report of the Working Group on *Crangon* fisheries and life history. ICES CM 2000/G:11, Ref. ACFM, B.
- ICES, 2006. Advice 2006. Answer to Special request on pulse trawl electrical fishinggear - Part I, 3 pp.
- ICES, 2006. Advice 2006. Answer to Special request on pulse trawl electrical fishinggear – Part II, 5 pp.
- ICES, 2012. Report of the Working Group on Crangon Fisheries and Life History (WGCRAN), ICES CM 2012/SSGEF:09. 79pp.
- ICES, 2013. Report of the working group on Crangon fisheries and life history (WGCRAN). ICES CM 2013/SSGEF, vol 12
- ICES, 2013b. Report of the Workshop on the Necessity for Crangon and Cephalopod Management (WKCCM), 8-9 October 2013, Copenhagen, Denmark. ICES CM 2013/ACOM:82
- ICES, 2014a. ICES Request from Germany and the Netherlands on the potential need for a management of brown shrimp (*Crangon crangon*) in the North Sea. Special Request, ICES Advice 2014, book 6.

- ICES, 2014b. Report of the Working Group on Beam Trawl Surveys (WGBEAM), 6-9 May 2014 Hamburg, Germany. ICES CM 2014/SSGESST:09
- ICES. 2015. Report of the Working Group on Crangon Fisheries and Life History (WGCRAN), 18–20 May 2015, Ijmuiden, the Netherlands. ICES CM 2015/SSGEPD:07. 58 pp.
- ICES. 2016. Interim Report of the Working Group on Crangon Fisheries and Life His-tory (WGCRAN), 23–25 May 2016, Oostende, Belgium. ICES CM 2016/SSGEPD:07. 33 pp.
- ICES, 2017. Report of the Working Group on Assessment of Demersal Stocks in the North Sea and Skagerrak (WGNSSK). ICES CM 2017/ACOM:21
- Jeffery, S., Revill, A., 2002. The vertical distribution of southern North Sea *Crangon crangon* (brown shrimp) in relation to towed fishing gears as influenced by water temperature. Fish Res. 55, 19–323.
- Jennings, S. and Kaiser, M. J. 1998. The effects of fishing on marine ecosystems. Advances in Marine Biology, 34: 221–233.
- Jordan, L. K., Mandelman, J. W., McComb, D. M., Fordham, S. V., Carlson, J. K. and Werner, T. B. 2013. Linking sensory biology and fisheries bycatch reduction in elasmobranch fishes: a review with new directions for research. Conservation Physiology, 1
- Jordan, L. J., Mandelman, J. W. and Kajiura, S. M. 2011. Behavioral responses to weak electric fields and a lanthanide metal in two shark species. J Exp Mar Biol Ecol 409: 345–350.
- Kaiser, M. J., Clarke, K. R., Hinz, H., Austen, M. C. V., Somerfield, P. J., and Karakassis, I. 2006. Global analysis and prediction of the response of benthic biota and habitats to fishing. Marine Ecology Progress Series, 311: 1–14.
- Kaiser, M.J., Attrill, M.J., Jennings, S., Thomas, D.N., Barnes, D.K.A., Brierley, A.S., Polunin, N.V.C., Raffaelli, D.G., Williams, P.J. 2005. Marine Ecology: Processes, Systems, and Impacts. Oxford University Press
- Kaiser, M., Ramsay, K., Richardson, C., Spence, F. and Brand, A. 2000. Chronic fishing disturbance has changed shelf sea benthic community structure. Journal of Animal Ecology, 69: 494-503.
- Kajiura, S. M., Cornett, A. and Yopak, K. 2010. Sensory adaptations to the environment: electroreceptors as a case study. In J Carrier, M Heithaus, J Musick, eds, Sharks and Their Relatives: Physiological Adaptations, Behavior, Ecology, Conservation, and Management. CRC Press, London, pp. 393–433.
- Kajiura, S. M. and Holland, K. N. 2002. Electroreception in juvenile scalloped hammerhead and sandbar sharks. J Exp Biol 205: 3609–3621.

- Kalmijn, A. J. 1971. The Electric Sense of Sharks and Rays. Journal of experimental biology, 55: 371-&.
- Kalmijn, A. J. 1972. Bioelectric fields in sea water and the function of the ampullae of lorenzini in elasmobranch fishes. SIO Reference, Scripps Institution of Oceanography, UC San Diego.
- Kalmijn, A., J. 1974. The detection of electric fields from inanimate and animate sources other than electric organs. In: Handbook of Sensory Physiology (Ed. by Fessard, A.), pp. 147-200. New York: Springer Verlag.
- Kalmijn, A. J. 1978. Electric and Magnetic Sensory World of Sharks, Skates, and Rays. Sensory Biology of Sharks, Skates, and Rays: 507-528.
- Kalmijn, A. J. 2000. Detection and processing of electromagnetic and near-field acoustic signals in elasmobranch fishes. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences, 355: 1135-1141.
- Keefe, M., Whitesel, T. A., and Angelone, P. 2000. Induced mortality and sub-lethal injuries in embryonic brook trout from pulsed DC electroshocking. North American Journal of Fisheries Management, 20: 320–327.
- Kempster, R. M., Hart, N. S. and Collin, S. P. 2013. Survival of the Stillest: Predator Avoidance in Shark Embryos. PLoS ONE 8(1): e52551. doi:10.1371/journal.pone.0052551
- Key 2014. Fish do not feel pain and its implications for understanding phenomenal consciousness. Biology & Philosophy, 30:2 p149-165
- Kimber, J. A, Sims D. W., Bellamy P. H. and Gill A. B. 2009. Male-female interactions affect foraging behaviour within groups of small-spotted catshark, Scyliorhinus canicula. Anim Behav 77:1435–1440
- Kimber, J. A., Sims, D.W., Bellamy, P. H. and Gill, A. B. 2011. The ability of a benthic elasmobranch to discriminate between biological and artificial electric fields. Mar Biol 158(1):1–8
- Kimber, J. A., Sims, D. W., Bellamy, P. H. and Gill, A. B. 2013. Elasmobranch cognitive ability: using electroreceptive foraging behaviour to demonstrate learning, habituation and memory in a benthic shark. Anim. Cogn. DOI 10.1007/s10071-013-0637-8
- Kjørsvika, E., Hoehne-Reitana, K., Reitanb, K.I., 2003. Egg and larval quality criteria as predictive measures for juvenile production in turbot (Scophthalmus maximus L.). Aquaculture 227 (2003) 9 –20.
- Klimley, A. P. 1993. Highly directional swimming by the scalloped hammerhead sharks, Sphyrna lewini, and subsurface irradiance, temperature, bathymetry and geomagnetic field. Marine Biology, 117, 1-22.

- Kolz, A. L., and Reynolds, J. B. 1990. Principles and techniques of electrofishing. Outline manual for course, Leetown, US, Fish and Wildlife Service Fisheries Academy.
- Kraan M., Trapman B.K.., Rasenberg M.M.M. 2015. Perceptions of European stakeholders of pulse fishing. IMARES Report number C098/15
- Kratzer I. 2012. Pulse beam trawling vs. traditional beam trawling in German shrimp fishery: a comparative study. Master thesis, Universität Rostock, Germany.
- Kraus, J. D. and Fleisch, D. A. 1999. Electromagnetics with applications. McGraw-Hill International Editions, Singapore
- Kynoch, R. J., Fryer, R. J. and Neat, F. C. 2015. A simple technical measure to reduce bycatch and discard of skates and sharks in mixed-species bottom-trawl fisheries. ICES Journal of Marine Science; doi:10.1093/icesjms/fsv037
- Lamarque, P. 1990. Electrophysiology of fish in electric fields. In: Fishing with Electricity, Applications in Freshwater Fisheries (eds I.G.L. Cowx and P. Lamarque). Fishing News Books, Blackwell Scientific Publications, Oxford, pp. 4–33.
- Last, K., Hendrick, V., Sotheran, I., Foster-Smith, B., Foster-Smith, D., and Hutchison, Z. 2012. Assessing the Impacts of Shrimp Fishing on Sabellaria spinulosa Reef and Associated Biodiversity in the Wash and North Norfolk SAC, Inner Dowsing Race Bank North Ridge SAC and Surrounding Areas. Report for Natural England
- Lindeboom, H. J. and de Groot, S. J. 1998. The effects of different types of fisheries on the North Sea and Irish Sea benthic eco-systems. EU-project AIR2-CT94-1664(IMPACT-II), Final Report ISSN 0923-3210, 404 pp.
- Logue, J.A., Howell, B.R., Bell, J.G. and Cossins, A.R. 2000. Dietary n-3 long-chain polyunsaturated fatty acid deprivation, tissue lipid composition, ex vivo prostaglandin production, and stress tolerance in juvenile Dover sole (*Solea solea* L.). Lipids 35, 745-55.
- Lough, R. G., Valentine, P. C., Potter, D. C., Auditore, P. J., Bolz, G. R., Neilson, J. D., and Perry, R. I. 1989. Ecology and distribution of juvenile cod and haddockin relation to sediment type and bottom currents on eastern Georges Bank. Marine Ecology Progress Series, 56: 1-12.
- Lund, I., 2007. The impact of dietarty fatty acids in common sole larval (*Solea solea* L.) nutrition. PhD thesis, Roskilde university.

- McGowan, D. W. and Kajiura, S. M. 2009. Electroreception in the euryhaline stingray, Dasyatis sabina. J Exp Biol 212: 1544–1552.
- Malkin S.Y., Meysman F.J.R. 2015. Rapid redox signal transmission by “cable bacteria” beneath a photosynthetic biofilm. Appl Environ Microbiol 81:948–956. doi:10.1128/AEM.02682-14
- Markle, D. F., and Frost. L. A. 1985. Comparative morphology, seasonality, and a key to the planktonic fish eggs from the Nova Scotian shelf. Canadian Journal of Zoology, 63: 246-257.
- Maksimov, Y., Malevicius, S., Yudin, V., 1987. Selective effect of electrified field while fishing by electrotrawl. Acta Hydrobiologica Lituanica 6, 54–74, ISSN 0208-2527.
- McBary, B. M. 1956. The effect of electric fields on marine fishes. In Scot. Home Dept.
- Meier, S., Morton, H. C., Nyhammer, G., Grøsvik, B. E., Makhotin, V., Geffen, A., Boitsov, S., Kvestad, K. A., Bohne-Kjersem, A., Goksøyr, A., Folkvord, A., Klungsøyr, J., and Svardal, A. 2010. Development of Atlantic cod (*Gadus morhua*) exposed to produced water during early life stages: Effects on embryos, larvae, and juvenile fish Marine Environmental Research, 70: 383-394.
- Meire, P., Ysebaert, T., Van Damme, S., Van den Bergh, E., Maris, T. & Struyf E. 2005. The Scheldt estuary: a description of a changing ecosystem Hydrobiologia (2005) 540:1–11
- Mellergaard, S & Nielsen, E. 1995. Impact of oxygen deficiency on the disease status of common dab Limanda limanda. Diseases of Aquatic Organisms 22, 101-114.
- Mellergaard, S & Nielsen, E. 1997. Epidemiology of lymphocystis, epidermal papilloma and skin ulcers in common dab Limanda limanda along the west coast of Denmark. Diseases of Aquatic Organisms 30(2), 151-163
- Mellergaard S, Bagge O. 1998. Fishing gear-induced skin ulcerations in Baltic cod, Gadus morhua L. Journal of Fish Diseases 21, 205-213.
- Molina, J. M. and Cooke, S. J. 2012. Trends in shark bycatch research: current status and research needs. Reviews in Fish Biology and Fisheries, 22: 719–737.
- Monk, J., Puvanendran, V., and Brown, J. A. 2006. Do different light regimes affect the foraging behaviour, growth and survival of larval cod (*Gadus morhua* L.)? Aquaculture 257: 287-293.
- Montgomery, J. and Walker, M. 2001. Orientation and navigation in elasmobranchs: which way forward? Environ Biol Fishes 60: 109–116.
- Morely, A. and Hughes, E. (Revised by Bolton, W.) 1994. Principles of Electricity, Fifth edition.

- Morgan, M.J., Wilson, C.E., and Crim, L.W. 1999. The effect of stress on reproduction in Atlantic cod. Journal of Fish Biology, 54: 477-488.
- Munk, P., Wright, P. J. and Pihl, N. J. 2002. Distribution of the Early Larval Stages of Cod, Plaice and Lesser Sandeel across Haline Fronts in the North Sea. Estuarine, Coastal and Shelf Science, 55: 139–149.
- Muth, R. T., and Rupert, J. B. 1997. Effects of electrofishing fields on captive embryos and larvae of razorback sucker. North American Journal of Fisheries Management, 17: 160–166.
- Neil, D.M., Ansell, A.D., 1995. The orientation of tail-flip escape swimming in decapod and mysid crustaceans. J. Mar. Biol. 75, 55–70.
- Neudecker, T., and Damm, U. 2010. The by-catch situation in German brown shrimp (*Crangon crangon* L.) fisheries with particular reference to plaice (*Pleuronectes platessa* L.). Journal of Applied Ichthyology, 26: 67-74.
- Nicolas D, Rochette S, Llope M, Licandro P 2014. Spatio-Temporal Variability of the North Sea Cod Recruitment in Relation to Temperature and Zooplankton. PLoS ONE 9(2): e88447. doi:10.1371/journal.pone.0088447
- Nikolakakis, S., Bossier, P., Kanlis, G., Dierckens K., and Adriaens D. 2014. Protocol for quantitative shape analysis of deformities in early larval European seabass *Dicentrarchus labrax*. Journal of Fish Biology, 84: 206–224.
- Nixon S.W. (1981) Remineralization and Nutrient Cycling in Coastal Marine Ecosystems. In: Neilson B.J., Cronin L.E. (eds) Estuaries and Nutrients. Contemporary Issues in Science and Society. Humana Press
- Noga, E.J., Udomkusonsri, P., 2002. Fluorescein: a rapid, sensitive, nonlethal method for detecting skin ulceration in fish. Vet. Pathol. 39, 726–731.
- Normandeau, Exponent, Tricas, T. and Gill, A. 2011. Effects of EMFs from Undersea Power Cables on Elasmobranchs and Other Marine Species. U.S. Dept. of the Interior, Bureau of Ocean Energy Management, Regulation, and Enforcement, Pacific OCS Region, Camarillo, CA. OCS Study BOEMRE 2011-09.
- Novotny, D.W. 1990. Electric fishing apparatus and electric fields. In: Fishing with Electricity, Applications in Fresh Water Fisheries Management. pp. 34-88. Cowx IG and Lamarque P (eds.). Oxford, England: Fishing News Books.

- Nutile, S., Amberg, J. J., Sepulveda, M. S., and Goforth R. R. 2012. Effects of multiple electrical field exposures on Cyprinid embryo survival. North American journal of fisheries management, 32: 875-879.
- Opstad, I., Suontama, J., Langmyhr, E., and Olsen, R. E. 2006. Growth, survival, and development of Atlantic cod (Gadus morhua L.) weaned onto diets containing various sources of marine protein. ICES Journal of Marine Science, 63: 320-325.
- Palazzi, R., Richard, J., Bozzato, G., and Zanella L. 2006. Larval and juvenile rearing of common sole (*Solea solea* L.) in the Northern Adriatic (Italy). Aquaculture, 255: 495-506.
- Paschen, M., Richter, U. and Köpnick, W. 2000. TRAPESE—Trawl Penetration in the Seabed. Final Report EU Contract 96-006, University of Rostock.
- Paulin, M. G. 1995. Electroreception and the Compass Sense of Sharks. Journal of Theoretical Biology, 174: 325-339.
- Pedersen, T., and Falk-Petersen, I. B. 1992. Morphological changes during metamorphosis in cod (Gadus morhua L.), with particular reference to the development of the stomach and pyloric caeca. Journal of Fish Biology, 41: 449-461.
- Peters, R. C., Eeuwes, L. B. and Bretschneider, F. 2007. On the electrodetection threshold of aquatic vertebrates with ampullary or mucous gland electroreceptor organs. Biol Rev Camb Philos Soc, 82: 361-373.
- Peters, R. C. and Evers, H. P. 1985. Frequency selectivity in the ampullary system of an elasmobranch fish (*Scyliorhinus canicula*) J. exp. Biol. 118, 99-109.
- Pfeffer C., Larsen S.,Song J., Dong M., Besenbacher F., Meyer R.L., Kjeldsen K.U., Schreiber L., Gorby Y.A., El-Naggar M.Y., Leung K.M., Schramm A., Risgaard-Petersen N. & Nielsen L.P. 2012. Filamentous bacteria transport electrons over centimetre distances. Nature VO L 4 9 1 - 2 1 9.
- Piccinetti, C. C., Ricci, L. A., Tokle, N., Radaelli, G., Pascoli, F., Cossignani, L., Palermo, F., Mosconi, G., Nozzi, V., Raccanello, F., and Olivotto I. 2012. Malnutrition may affect common sole (*Solea solea* L.) growth, pigmentation and stress restpnse: Molecular, biochemical and histological implications. Comparative biochemistry and physiology, Part A 161: 361-371.
- Piet, G. J., Rijnsdrop, A. D., Bergman, M. J. N. and van Santbrink, J. 2000. A quantitative evaluation of the impact of beam trawling on benthic fauna in the southern North Sea. ICES Journal of Marine Science: Journal du Conseil, 57: 1332–1339.

- Piet, G. J., and N. T. Hintzen. 2012. Indicators of fishing pressure and seafloor integrity. ICES Journal of Marine Science 69: 1850–1858.
- Polet, H. 2000. Codend and whole trawl selectivity of a shrimp beam trawl used in the North Sea. Fisheries Research, 48: 167-183.
- Polet, H. 2002. Selectivity experiments with sorting grids in the North Sea brown shrimp (*Crangon crangon*) fishery. Fisheries Research, 54: 217-233.
- Polet, H. 2003. Evaluation of by-catch in the Belgian brown shrimp (*Crangon crangon* L.) fishery and of technical means to reduce discarding. PhD thesis, Ghent University.
- Polet, H., Delanghe, F. and Verschoore, R. 2005a. On electrical fishing for brown shrimp (*Crangon crangon*): I. Laboratory experiments. Fisheries Research, 72: 1-12.
- Polet, H., Delanghe, F. and Verschoore, R. 2005b. On electrical fishing for brown shrimp (*Crangon crangon*): II. Sea trials. Fisheries Research, 72: 13-27.
- Polet, H., and Depestele, J. 2010. Impact assessment of the effects of a selected range of fishing gears in the North Sea. ILVO-report. Oostende, Belgium. 120 pp.
- Polet, H., 2010. Electric senses of fish and its application in marine fisheries. In He, P. (ed.), Behavior of marine fishes: capture processes and conservation challenges, 1 edn. Wiley-Blackwell, Iowa, , pp 205-235.
- Puvanendran, V., and Brown, J. A. 1999. Foraging, growth and survival of Atlantic cod larvae reared in different prey concentrations. Aquaculture, 175: 77–92.
- Puvanendran, V., and Brown, J. A. 2002. Foraging, growth and survival of Atlantic cod larvae reared in different light intensities and photoperiods. Aquaculture, 214: 131-151.
- Quijrijns, F., Strietman, W. J., van Marlen, B., Rasenberg, M., and Smith, S. R. 2015. Flatfish pulse fishing. Research results and knowledge gaps II. Report C091/15. 39 pp.
- Rasenberg, M., van Overzee, H., Quirijns, F., Warmerdam, M., van Os, B. and Rink, G. 2013. Monitoring catches in the pulse fishery Wageningen, IMARES: 59p.
- Revill, A., Pascoe, S., Radcliffe, C., Riemann, S., Redant, F., Polet, H., Damm, U., et al. 1999. The economic consequences of discarding in the Crangon fisheries. Final report to the European Commission, 118.
- Revill, A. S., and Holst, R. 2004. Reducing discards of North Sea brown shrimp (*C. crangon*) by trawl modification. Fisheries Research, 68: 113-122.

- Revill, A., Pascoe, S., Radcliffe, C., Riemann, S., Redant, F., Polet, H., Damm, U., Neudecker, T., Kristensen, P. and Jensen, D., 1999. The economic & biological consequences of discarding in the European Crangon fisheries. Final report to the European Commission, Contract No. 97/SE/025.
- Rijnsdorp, A., de Haan D., Smith, S., Strietman W.J. 2016. Pulse fishing and its effects on the marine ecosystem and fisheries. An update of the scientific knowledge. Wageningen University & Research Report C117/16
- Rijnsdorp, A.D., Van Beek, F.A., Flatman, S., Millner, R.M., Riley, J.D., GIRET, M. and De Clerck, R. 1992. Recruitment of sole stocks, *Solea solea* (L.) in the Northeast Atlantic. Netherlands Journal of Sea Research 29: 1-3
- Roach, S. M. 1999. Influence of electrofishing on the mortality of Arctic grayling eggs. North American Journal of Fisheries Management, 19: 923–929.
- Rollefsen, G. 1930. The Susceptibility of Cod Eggs to external Influences. Norwegian Bureau of Fisheries, Bergen.
- Roman, M.R., Ducklow, H.W., Fuhrman, J.A., Garside, C., Glibert, P.M., Malone, T.C., McManus, G.B. 1988. Marine Ecology – Prog. Ser. 42:39-52
- Rose J.D., Arlinghaus, R., Cooke, S.J., Diggles, B.K., Sawynok, W., Stevens E.D. and Wynne, D.D.L. 2014. Can fish really feel pain? Biology & Philosophy Volume 30, Issue 2, pp 149–165
- Rose, J.D. 2002. The neurobehavioral nature of fishes and the question of awareness and pain. Reviews in Fisheries Science 10, 1–38.
- Scheminzky F. 1924. Versuche über Electrotaxis und Electronarkose. Pflügers Arch. ges. Physiol. 202: 200–216.
- Schreer, J.F., S.J. Cooke, K.B. Connors. 2004. Electrofishing-induced cardiac disturbance and injury in rainbow trout. Journal of Fish Biology 64: 996-1014.
- Scientific, Technical and Economic Committee for Fisheries (STECF) 2014. Evaluation of Fishing Effort Regimes in European Waters - Part 2. Publications Office of the European Union, Luxembourg, EUR 27027 EN, JRC 93183, 8 44 pp.
- Scientific, Technical and Economic Committee for Fisheries (STECF) 2014b. Landing Obligations in EU Fisheries - part 3. Publications Office of the European Union, Luxembourg, EUR 26610 EN, JRC 89785, 56

pp. Evaluation of Fishing Effort Regimes in European Waters - Part 2. Publications Office of the European Union, Luxembourg, EUR 27027 EN, JRC 93183, 8 44 pp.

- Scientific, Technical and Economic Committee for Fisheries (STECF) 2015 – Technical Measures part 3. Publications Office of the European Union, Luxembourg, EUR 27223 EN, JRC 95832, 59 pp.
- Seidel WR and Watson JW. 1978. A trawl design: employing electricity to selectively capture shrimp. MFR Pap. 1325: 21–23.
- Sharber, N.G. and Black, J.S. 1999 Epilepsy as a unifying principle in electrofishing theory. a proposal. Trans. Am. Fish. Soc. 128: 666–671.
- Sisneros, J. A., Tricas, T. C. and Luer, C. A. 1998. Response properties and biological function of the skate electrosensory system during ontogeny. Journal of Comparative Physiology A, 183, 87-99.
- Smaal, A. C. and Brummelhuis, E. 2005. Onderzoek naar mogelijke effecten van de pulskor op bodemdieren. ICES Document C089/05. 15 pp.
- Snyder, D.E. 2003. Electrofishing and its harmful effects on fish. U.S. Geological Survey, Information and Technology Report 2003-0002.
- Soetaert, M., Chiers, K., Duchateau, L., Polet, H., Verschueren, B. and Decostere, A. 2014. Determining the safety range of electrical pulses for two benthic invertebrates: brown shrimp (*Crangon crangon* L.) and ragworm (*Alitta virens* S.). ICES Journal of Marine Science: Journal du Conseil 72: 973-980.
- Soetaert, M., Decostere, A., Polet, H., Verschueren, B. and Chiers, K. 2015. Electrotrawling: a promising alternative fishing technique warranting further exploration. Fish and Fisheries, 16: 104-124.
- Soetaert M. 2015 Electrofishing: exploring the safety range of electric pulses for marine species and its potential for further innovation. Ghent University PhD Thesis
- Soetaert, M., Decostere, A., Verschuren, B., Saunders, J., Van Caelenberge, A., Puvanendran, V., Mortensen, A., Duchateau, L., Polet, H. & Chiers, K., 2016a. Side-effects of electrotrawling: exploring the safe operating space for Dover sole (*Solea solea* L.) and Atlantic cod (*Gadus morhua* L.). Fisheries Research 177, 95-103
- Soetaert, M., De Haan, D., Verschueren, B., Decostere, A., Puvanendran, V., Saunders, J., Polet H. and Chiers K. 2016b. Atlantic Cod Show a Highly Variable Sensitivity to Electric-Induced Spinal Injuries. Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science 8:412–424, 2016

- Soetaert, M., Verschueren, B., Chiers, K., Duchateau, L., Polet H. & Decostere A. 2016c. Laboratory Study of the Impact of Repetitive Electrical and Mechanical Stimulation on Brown Shrimp *Crangon crangon*. Marine and Coastal Fisheries Vol. 8 , Iss. 1
- Speare, D.J., and H.W. Ferguson. 2006. Gills and pseudobranchs. In: Ferguson, H.W. (ed.), Systemic Pathology of Fish, 2 edn. Scotian Press, London, UK, pp. 24-64.
- Sreedharan N, Vijayan V and Hridayanathan C. 1977. Development of electro-trawl system in marine environment. Fish. Technol. 14, No 1.
- Stappenbeck, F. 2017. Investigation of the reaction of brown shrimp on pulsed electric fields in order to optimise Crangon pulse trawls. Master thesis at Rostock University, 68 p.
- Steins, N.A., Smith, S., Strietman, W.J., Trapman, B.,Kraan, M., 2017. International Stakeholder Dialogue on Pulse Fisheries; Report of the second dialogue meeting, Amsterdam 20 January 2017. Wageningen, Wageningen Marine Research (University & Research centre), Wageningen Marine Research report C016/17, 45 pp.
- Stentiford, G.D., J.P. Bignell, B.P. Lyons, J.E. Thain, S.W. Feist. 2010. Effect of age on liver pathology and other diseases in flatfish: implications for assessment of marine ecological health status. Marine Ecology Progress Series 411: 215-230.
- Stepputtis, D., Zajicek, P., Vorberg, R., Berkenhagen, J., and Kratzer, I. 2014. Ökologische und ökonomische Untersuchungen zum Nutzen einer Pulsbaumkurre in der deutschen Garnelenfischerei (Projektbericht); 231 pp.
- Sternin, Nikonorov, Bumeister, 1972. Electrical Fishing, Theory and Practice. Pishchevaya Promyshlennost, Moskva, 1972. Translated from Russian: Israel Program for Scientific Translations,
- Stewart, P. A. M. 1972. The selection of electrode materials for electrical fishing. ICES Document IR 72–1. 12 pp.
- Stewart, P. A. M. 1975. Catch selectivity by electrical fishing systems. ICES Journal of Marine Science: Journal du Conseil, 36: 106–109.
- Stewart, P. A. M. 1977. A study of the response of flatfish (Pleuronectidae) to electric stimulation. ICES Journal of Marine Science: Journal du Conseil, 37: 123–129.
- Stewart, M. E., Blogoslawski, W. J., Hsu, R. Y., Helz, G. R. 1979. By-products of oxidative biocides: Toxicity to oyster larvae. Marine pollution bulletin, 10: 166-169.

- Stiasny M.H., Mittermayer F.H., Sswat M., Voss R., Jutfelt F., Chierici M., Puvanendran V., Mortensen A., Reusch T.B.H. and Clemmesen C. 2016. Ocean Acidification Effects on Atlantic Cod Larval Survival and Recruitment to the Fished Population. PLOS ONE | DOI:10.1371/journal.pone.0155448
- Stroup, W. 2012. Generalized Linear Mixed Models: Modern Concepts, Methods and Applications. CRC Press.
- Sundby, S. 1983. A one-dimensional model for the vertical distribution of pelagic fish eggs in the mixed layer. Deep-Sea Research, 30: 645-661.
- Suuronen, P., Chopin, F., Glass, C., Løkkeborg, S., Matsushita, Y., Queirolo, D., and Rihan, D. 2012. Low impact and fuel efficient fishing—Looking beyond the horizon. Fisheries Research, 119: 135-146.
- Sys, K., Poos, J. J., Van Meensel, J., Polet, H., and Buysse, J. 2016. Competitive interactions between two fishing fleets in the North Sea. ICES Journal of Marine Science, 73 1485 -1493
- Teal, L. R., Depestele, J., O'Neill, B., Craeymeersch, J. A. M., Denderen, P. D., Parker, R., Perdon, K. J., et al. 2014. Effects of beam and pulse trawling on the benthic ecosystem. ICES Document C098/14. 53 pp.
- Temming, A., and Hufnagl, M. 2015. Decreasing predation levels and increasing landings challenge the paradigm of non-management of North Sea brown shrimp (*Crangon crangon*). ICES Journal of Marine Science, 72: 804–823.
- Tiews, K. 1970. Synopsis of biological data on the common shrimp *Crangon crangon* (Linnaeus, 1758). FAO Fish. Synop. 57, 1167-1224
- Thorsen, A., Trippel, E., and Lambert Y. 2003. Experimental methods to monitor the production and quality of eggs of captive marine fish. Journal of Northwest Atlantic Fisheries science, 33: 55-70.
- Tilseth, S., Solberg, T.S., and Westrheim, K. 1984. Sublethal effects of the water-soluble fraction of Ekofisk crude oil on early larval stages of cod (*Gadus morhua* L.). Marine Environmental Research, 11: 1-16.
- Tricas, T. C. and Sisneros, J. A. 2004. Ecological functions and adaptations of the elasmobranch electrosense. In: The Senses of Fish: Adaptations for the Reception of Natural Stimuli (Ed. by G. von der Emde), pp. 308–329. New Delhi: Narosa Publishing.
- Tricas, T. C. 2001. The neuroecology of the elasmobranch electrosensory world: Why peripheral morphology shapes behavior. Environmental Biology of Fishes, 60: 77-92.
- Tricas, T.C., and New, J.G. 1998. Sensitivity and response dynamics of elasmobranch electrosensory primary afferent neurons to near threshold fields. J. Comp. Physiol. 182: 89-101.

- Tricas, T. C., Michael, S. W. and Sisneros, J. A. 1995. Electrosensory optimization to conspecific phasic signals for mating. Neuroscience Letters, 202: 129-132.
- Tulp, I., Chen, C., Haslob, H., Schulte, K., Siegel, V., Steenbergen J., Temming, A. and Hufnagl, M. 2016. Annual brown shrimp (*Crangon crangon*) biomass production in Northwestern Europe contrasted to annual landings. ICES J. Mar. Sci. 2016: fsw141v1-fsw141.
- Turenhout, M.N.J., B.W. Zaalmink, W.J. Strietman, K.G. Hamon, 2016. Pulse fisheries in the Netherlands; Economic and spatial impact study. Wageningen Economic Research, Report 2016-104. 32 pp.
- Uhlman, S., Theunynck, R., Desender, M., Soetaert, M. and Depestele, J. 2016. Reflex impairment of beam-trawled flatfish. ICES Journal of Marine Science, Volume 73, Issue 4.
- Urzúa, Á., and Anger, K. 2013. Seasonal variations in larval biomass and biochemical composition of brown shrimp, *Crangon crangon* (Decapoda, Caridea), at hatching. Helgoland Marine Research, 67: 267-277.
- van Denderen, P. D., Hintzen, N. T., van Kooten, T., and Rijnsdorp, A. D. 2015. Temporal aggregation of bottom trawling and its implication for the impact on the benthic ecosystem. – ICES Journal of Marine Science, 72: 952–961.
- van der Reijden, K.J., Molenaar, P., Chen, C., Uhlmann S.S., Goudswaard, P.C. and van Marlen, B. 2017. Survival of undersized plaice (*Pleuronectes platessa*), sole (*Solea solea*), and dab (*Limanda limanda*) in North Sea pulse-trawl fisheries. ICES Journal of Marine Science, 74(6), 1672–1680. doi:10.1093/icesjms/fsx019
- Van der Veer, H.W., Dapper, R., Witte, J.I.J., 2001. The nursery function of the intertidal areas in the western Wadden Sea for 0-group sole *Solea solea* (L) J. Sea Res., 45 (2001), pp. 271-279
- van Marlen, B., 1997. Alternative stimulation in fisheries. Final report contract no AIR3-CT94-1850.190 pp.
- van Marlen B., F. Redant, H. Polet, C. Radcliffe, A. Revill, P.S. Kristensen, K.E. Hansen, H.J. Kuhlmann, S. Riemann, Th. Neudecker, and J.C. Bradant. 1998. Research into Crangon Fisheries Unerring Effect. (RESCUE) - EU-Study 94/044. RIVO Report C054/97.
- van Marlen, B., de Haan, D., van Gool, A. and Burggraaf, D. 2009. The effect of pulse stimulation on marine biota – Research in relation to ICES advice – Progress report on the effects on benthic invertebrates. ICES Document C103/09. 53 pp.
- van Marlen, B., Wiegerinck, J., van Os-Koomen, E. and van Barneveld, E. 2014. Catch comparison of flatfish pulse trawls and a tickler chain beam trawl. Fisheries Research, 151: 57-69.

- van Stralen, M. R. 2005. De Pulskor. MarinX-rapport 2005.26. 26 pp. Vibert, R. 1963. Neurophysiology of electric fishing. Transactions of the American Fisheries Society, 92: 265–275.
- Vercauteren, M, Declercq, A, De Swaef, E, Devriese, L, Polet, H, Decostere, A & Chiers, K 2016, Role of Vibrio tapetis in the development of skin ulceration in common dab (Limanda limanda). in BOOK OF ABSTRACTS. vol. VLIZ Special publication 75, Vlaams Instituut voor de Zee, blz. 1-165.
- Verschueren, B. and Polet, H. 2009. Research summary on HOVERCRAN, hovering pulse trawl for a selective *Crangon* fishery. Institute for Agricultural and Fisheries Research, Report nr.: 23.
- Verschueren, B., Vanelslander, B. and Polet, H. 2012. Verduurzaming van de Garnalenvisserij met de Garnalenpuls. Institute for Agricultural and Fisheries Research, Report nr.: 116. 102pp.
- Verschueren, B., and Vanelslander B., 2013. Project Combipuls: Pulsvisserij op tong en garnaal. Institute for Agricultural and Fisheries Research. 74pp.
- Verschueren B., Lenoir H., Vandamme L. and Vanelslander B., 2014. Evaluatie van een seizoen pulsvisserij op garnaal met HA 31. Institute for Agricultural and Fisheries Research, Report nr.: 157. 104pp.
- Verschueren B., and Lenoir H., 2016 De pulsvisserij op garnalen: vergelijking met een klassiek vistuig. ILVO mededeling 211 ISSN 1784-3197
- Verweij, M., van Densen, W., 2010. Differences in causal reasoning about resource dynamics and consequences for the participatory debate on North Sea fisheries. Marine Policy Mar. Pol. 34, 1144–1155.
- Vibert, R. 1967. in Fishing with electricity – its applications to biology and management Contributions to a Symposium (Vibert, R., editor). General Report of the Working party on the Applications of Electricity to Inland Fishery Biology and Management, Fishing News (Books) Ltd, London.
- Walsh, S.J., Engas, A., Ferro, R.S.T., Fonteyne, R. and Van Marlen, B., 2000. Improving fishing technology to catch (or conserve) more fish: the evolution of the ICES Fishing Technology and Fish Behaviour Working Group during the past century. ICES Symposium on 100 years of science under ICES, 1-4 Finland.
- Walve, J., Larsson, U. 1999. Carbon, nitrogen and phosphorus stoichiometry of crustacean zooplankton in the Baltic Sea: implications for nutrient recycling. Journal of Plankton Research, 21: 12,1
- Welleman, H. C., and Daan, N. 2001. Is the Dutch shrimp fishery sustainable? Senckenbergiana Maritima, 31: 321–328.
- White, W. T., Blaber, S. J. M. and Craig, J. F. 2012. The current status of elasmobranchs: biology, fisheries and conservation. J Fish Biol, 80: 897-900.

- Wiklund T & Bylund G. 1993. Skin ulcer disease of flounder Platichthys flesus in the northern Baltic Sea. Diseases of aquatic organisms. 17, 165-174
- Yalçin, H., Koç, T., Pamuk, V. 1997. Hydrogen and bromine production from concentrated sea-water. Int. Journal of Hydrogen Energy, 22: 967-970
- Yamagami, K. 1988. Mechanisms of hatching in fish. In Fish Physiology, 11: 447–499.
- Yano, K., Mori, H., Minamikawa, K., Ueno, S., Uchida, S., Nagai, K., Toda, M. and Masuda, M. 2000. Behavioural response of sharks to electric stimulation. Bull Seikai Natl Fish Res Inst 78:13–29
- Yin, M. C. and Blaxter, J., H., S. 1987.Temperature, salinity tolerance, and buoyancy during early development and starvation of Clyde and North Sea herring, cod, and flounder larvae. J. Exp. Mar. Biol. Ecol., 107: 279-290.
- Yu, C., Chen, Z., Chen, L., and He, P. 2007. The rise and fall of electrical beam trawling for shrimp in the East China Sea: technology, fishery, and conservation implications. ICES Journal of Marine Science: Journal du Conseil, 64: 1592-1597.

Samenvatting

De niet aflatende vraag naar grijze garnaal, *Crangon crangon*, maakt van de **garnaalvisserij** in de Noordzee een **economisch belangrijke** activiteit. Met een vloot bestaande uit meer dan 500 vaartuigen wordt jaarlijks tot 35 000 ton van deze delicatesse aangevoerd (Verschueren et al., 2012; ICES, 2012). Desondanks kampt de huidige garnaalvisserij met enkele belangrijke **problemen**. Bodemsleepnetvisserijen kunnen heel wat bodemberoering veroorzaken enhoge hoeveelheden bijvangst en teruggooi genereren die slechts geringe overlevingskansen hebben. Bovendien gaat garnaalvisserij vaak door in kwetsbare kustgebieden en estuaria, kraamkamers voor vele dieren.

Het gebruik van **elektrische pulsen** voor het vangen van grijze garnaal of platvis wordt dezer dagen beschouwd als een veelbelovend alternatief bij het bouwen aan een **duurzamere visserij**. Tegenwoordig zijn er een kleine honderd schepen uitgerust met deze techniek in de zuidelijke Noordzee waarbij er twee verschillende typen kunnen onderscheiden worden. De meerderheid maakt gebruik van een platvispuls, voor het vangen van o.a. tong (*Solea solea*). Hierbij wordt de mechanische stimulatie van wekkers of kettingen gedeeltelijk vervangen door een kramppuls met hoge frequentie 60-80Hz. De puls zorgt voor een kramp in de tong waardoor deze moeilijker kan ontsnappen. Slechts een vijftal schepen maken gebruik van een schrikpuls bij het vangen van garnaal. Het concept van het gebruik van elektrische pulsen in de (garnaal)visserij wordt toegelicht in Hoofdstuk 1, waarbij ook de voordelen en hiaten in kennis omtrent deze techniek worden aangekaart.

Bij gebruik van de **garnaalpuls** is de mechanische stimulatie van de bobbijnroede voor het opschrikken van garnaal (gedeeltelijk) vervangen door 12 lichte elektroden die een elektrisch veld met lage frequentie, 5Hz, opwekken. Dit veld zorgt ervoor dat de garnaal selectief uit de bodem opspringt waarna deze gevangen wordt door een zwevend net. Andere organismen blijven ongemoeid en kunnen onder het net door ontsnappen. Pulsvisserij voor garnaal heeft bijgevolg een **gedaalde milieu-impact** wegens verminderd (tot 76%) bodemcontact en gereduceerde bijvangst en teruggooi (35-76%).

Er is echter bijzonder **weinig gekend over de impact van elektrische pulsen op mariene organismen**. Grondig onderzoek naar deze effecten is dan ook cruciaal om een re-evaluatie van de huidige ban op elektrisch vissen in de EU, een eventueel bredere commerciële introductie en vervolgens mogelijke certificering van dit type vistuig mogelijk te maken. De algemene doelstelling van het doctoraatsonderzoek (Hoofdstuk 2) is dan ook na te gaan of het gebruik van een garnaalpuls weldegelijk een ecologisch verantwoord alternatief biedt voor de traditionele garnaalvisserij.

Ondanks het feit dat eerder onderzoek naar de impact van de garnaalpuls op enkele **invertebraten** en **volwassen vis** geen directe effecten kon aantonen bleven toch nog heel wat vragen onbeantwoord. Zo werd het gedrag van de vissen tijdens en na de blootstelling niet systematisch geëvalueerd en werden geen RX-foto's genomen. Hierdoor konden mogelijke letsels ter hoogte van de ruggengraat, één van de belangrijkste bevindingen vastgesteld bij gebruik van de platvispuls in rondvis zoals kabeljauw, niet gedetecteerd worden. Alsook ontbrak een gedetailleerde zoektocht naar eventuele macroscopische als zowel microscopische letsels. In Hoofdstuk 3 werd een reeks mariene organismen die algemeen voorkomen in de bijvangst van garnaalkotters met name: tong (*Solea solea*), pladijs (*Pleuronectes platessa*), kabeljauw (*Gadus morhua*), harnasmannetje (*Agonus cataphractus)* en zeedonderpad (*Myoxocephalus scorpius*), blootgesteld tussen drie elektroden aan een heterogeen garnaalpuls elektrisch veld gedurende vijf seconden. De nodige controlegroepen werden ingesloten, waarbij de dieren op dezelfde manier als de blootgestelde dieren werden behandeld, maar zonder het aanleggen van een elektrisch veld. Gedurende een half uur (kort voor, tijdens en na de blootstelling) werd het gedrag gefilmd met een camera. Na 24 uur werden de vissen geëuthanaseerd en werd een autopsie uitgevoerd. Van kieuwen, dorsaal spierweefsel, hart, lever, milt, nier en darm werden stalen genomen voor histologisch onderzoek. Van elke vis werden eveneens RX-foto's genomen. Onder de condities zoals toegepast in onze studie kon slechts een geringe directe korte termijn impact van het elektrisch veld worden vastgesteld. Conform vorige studies overleefden alle organismen de blootstelling. Tevens kon er geen schade aan de ruggengraat worden vastgesteld. Effecten op het gedrag varieerden sterk en waren soort afhankelijk. Zo reageerde kabeljauw snel zwemmend en sterk geagiteerd op de pulsen tijdens blootstelling. Ook 15% van de tong zwom actief op gedurende de blootstelling. De meerderheid van de vissen bleven echter dicht bij de bodem en vertoonden een minimale reactie gedurende de hele observatie periode. Macroscopische bloedingen die voornamelijk in pladijs en tong werden vastgesteld waren niet significant verschillend tussen blootgestelde en controle groepen. Bij twee blootgestelde pladijzen werd een microscopisch kleine bloeding in de spier vastgesteld. Ook het aantal melanomacrofagen centers in de milt was significant hoger in blootgestelde kabeljauw.

Garnaalvisserij vindt vaak plaats in kwetsbare kustgebieden en estuaria, paaiplaatsen en kraamkamers voor vele diersoorten (Van Marlen et al., 1998). Verschillende studies suggereren dat pulsvissen op dergelijke plaatsen de embryonale en larvale levensstadia zouden kunnen beschadigen (Dwyer en Erdahl, 1995; Muth en Rupert, 1997; Roach, 1999; Henry en Grizzle, 2004; Bohl et al., 2010). Ook juveniele vis vlak na de metamorfose zou zeer kwetsbaar zijn (Henry en Grizzle, 2003). Literatuur over het effect van pulsvissen op **vroege levensstadia** is verder schaars en beperkt zich tot enkele zoetwaterorganismen en zalmachtigen (Lamarque 1990; Snyder, 2003). De impact op de verschillende levensstadia van mariene organismen is evenwel nog nooit onderzocht. Het was dan ook de bedoeling om mogelijke schadelijke effecten van de garnaalpuls op verschillende levensstadia van tong en kabeljauw in kaart te brengen. Vermits de embryonale, larvale en waarschijnlijk ook de juveniele levensstadia zeer dicht bij de elektroden kunnen komen, werd een hogere veldintensiteit van 150V/m uitgetest in een homogeen elektrisch veld waarbij de andere pulskarakteristieken identiek waren aan deze van de garnaalpuls. Daar adulte **kabeljauw** reeds gevoelig bleek aan de elektrische pulsen gebruikt in de platvisvisserij, getuige vastgestelde wervelletsels, werd in Hoofdstuk 4 onderzoek verricht met verschillende levensstadia van deze rondvis. Drie embryonale, vier larvale en één juveniel stadium werden blootgesteld aan elektrische pulsen gedurende vijf seconden. Er konden geen significante verschillen worden vastgesteld in de overleving van de embryonale stadia. Bij het embryonale stadium blootgesteld op 18 dagen na bevruchting werd in eerste instantie evenwel een lagere ontluikingsratio vastgesteld in vergelijking met de niet-blootgestelde dieren. Larven die blootgesteld waren 2 en 26 dagen na ontluiken vertoonden een significant hogere mortaliteit ten opzichte van de controle groepen. In de andere larvale en juveniele stadia kon geen impact op de overleving worden vastgesteld. Omdat mogelijke effecten niet noodzakelijk lethaal hoeven te zijn maar zich kunnen manifesteren in afwijkende groei en grootte werd een morfometrische analyse op de uitgekomen larvale en juveniele stadia uitgevoerd. Er konden echter ook hier geen verschillen worden vastgesteld in de snelheid van dooier resorptie, mogelijke misvormingen of de totale lengte, het oog, de kop en spierhoogte van de notochord. Omdat platvissen een zeer complexe ontwikkeling ondergaan, werd een gelijkaardige studie uitgevoerd op **tong** embryo's (2 dagen na bevruchting) en larven (11 dagen na ontluiken) (hoofdstuk 5). Er kon geen lagere overleving worden vastgesteld voor zowel blootgestelde eitjes als larven. Bovendien werden ook hier geen verschillen in de morfometrische metingen geobserveerd in de zich ontwikkelende larven op 6 en 19 dagen na uitkomen.

In Hoofdstuk 6 werd de prooidetectie van **elektrosensitieve dieren zoals haaien** onderzocht. Deze dieren sporen immers via elektrosensitieve organen (ampullen van Lorenzini) hun prooi op aan de hand van het elektrisch veld dat ze uitzenden. Blootstelling aan elektrische pulsen zou mogelijks het elektrosensorisch detectiesysteem van deze zeer kwetsbare kraakbeenvissen kunnen verstoren en zo hun foerageergedrag in het gedrang brengen. Voor deze proef werd de prooidetectie door de haai geverifieerd door middel van een gesimuleerd elektrisch veld gelijkaardig aan dat van het pooi dier. Deze elektrorespons werd vervolgens zowel voor als drie dagen na blootstelling aan het elektrisch veld gebruikt voor het vangen van garnaal en platvis geobserveerd. Er konden geen significante verschillen worden aangetoond in het aantal haaien dat een elektrorespons vertoonde voor en na blootstelling en tussen controle en blootgestelde groepen. Eveneens kon geen verschil in tijd tussen de start van het foerageer gedrag en de effectieve beet naar het gesimuleerde prooi elektrische veld worden vastgesteld.

In de algemene discussie (Hoofdstuk 7) worden de resultaten van de verschillende studies samengevat en in perspectief geplaatst. Tevens wordt gewezen op de noodzaak voor verder onderzoek met oplijsting van een aantal mogelijke pistes. Tot slot worden enkele problemen aangekaart.

Hoewel de directe impact van elektrische garnaalvisserij klein lijkt in vergelijking met deze van de traditionele garnaalboomkor heerst er nog heel wat bezorgdheid en onzekerheid. Tevens is verder onderzoek naar lange termijn effecten en andere componenten van het ecosysteem aangewezen teneinde een duurzame garnaalvisserij te verzekeren.

Summary

Brown shrimp are caught with bottom trawls, as is the case for 90% of all demersal fish, shell and crustacean landings in the North Sea. These demersal trawl fisheries are known to produce large amounts of by-catch and discards with low chances of survival and to disturb the seafloor habitat of benthic organisms. This results in severe ecosystem damage and the indirect reduction of quota in other fisheries. Furthermore, fuel consumption is high. In order to increase the sustainability of these fisheries and consider ecological certification, technical adaptations are necessary. Electric pulses in fishing gear are increasingly used in the North Sea and are considered a promising alternative to ameliorate the sustainability of demersal trawl fisheries. Two systems are currently used in almost 100 vessels. Most vessels use a flatfish pulse to chase in particular sole (*Solea solea*). Mechanical stimulation by chainmats and tickler chains to chase the flatfish is partly replaced by a high frequency pulse, 60-80Hz. This induces a cramp in flatfish, making them immobile and easier to catch. Five vessels equip their vessel with a shrimp pulse to catch brown shrimp (*Crangon crangon*). The basic principles of the use of electric pulses in fisheries are elaborated on in Chapter 1, whereby the advantages and knowledge gaps of this technique are listed.

When using a shrimp pulse, the bobbin rope is replaced by 12 light weight electrodes creating a low-intensity, 5Hz, electric field which selectively induces a startle response in the shrimps. Other benthic organisms are left untouched and can escape underneath the hovering trawl that collects the jumping shrimps without disturbing the seabed, engendering decreased environmental impact by reduced bottom contact (-76%) and decreased by-catch (-35-76%) (Verschueren et al., 2009).

Nevertheless, the effects of suchlike electric pulse field on marine organisms are largely unknown (Snyder 2003). The general aim of this research was therefore to assist in gathering scientific data to enable the provision of an answer to the question whether electric pulse trawling for brown shrimp is ecologically justifiable by assessing its possible harmful effects on various marine fish species and life

stages (Chapter 2). This information could enable making well-founded decisions on the possible lift the standing ban on electric fishing in the EU.

In first instance, short-term direct effects of this pulse used for electrotrawling for brown shrimp on five **adult** marine fish species inhabiting shrimp fishery areas were investigated in Chapter 3. For this purpose, European plaice (*Pleuronectes platessa*), Dover sole (*Solea solea*), Atlantic cod (*Gadus morhua*), bull-rout (*Myoxocephalus scorpius*) and armed bullhead (*Agonus cataphractus*) were exposed to the shrimp pulse for 5 s. Before, during and till 20 min following exposure, the behaviour of the fish was monitored. Twenty-four hours post-exposure, all fish were sacrificed, inspected and samples for histological analysis were taken from the gills, dorsal muscle and internal organs. To investigate possible spinal injuries radiographs were taken. Behavioural responses were variable and species dependent. Roundfish species, cod in particular, were displaying more active and agitated fast swimming activity during exposure. The majority of flatfish showed only minor reactions and remained close to the bottom throughout the observation period. However, 15% of the exposed sole actively swam upwards during exposure. Mild multifocal petechial haemorrhages and suffusion, encountered mainly in plaice and sole, were not significantly different between exposed and control groups. Upon histological examination, in two exposed plaice, a focal small haemorrhage between muscle fibers was found, which was not encountered in control animals. In addition, the number of melanomacrophage centres in the spleen of exposed cod was significantly higher than in the non-exposed animals. In conclusion, under the circumstances as adopted in this study, the electric field seemed to have only limited immediate short-term impact on the exposed animals.

As brown shrimp are caught in shallow coastal zones and estuaries, important nurseries or spawning areas for a wide range of marine species, electrotrawling on these grounds could therefore harm embryos, larvae and juveniles. However, the impact on **young marine life stages** was unknown at the initiation of the current research. In Chapter 4 experiments were carried out on different developmental stages of cod (*Gadus morhua*) which are considered vulnerable to electric pulses. Three embryonic, four larval and one juvenile stage were exposed to a homogeneous electric field of 150 V/m $_{peak}$ for 5 s mimicking a worst-case scenario. No significant differences in embryo mortality rate were found between control and exposed groups. However, in the embryonic stage exposed at 18 days post fertilization, the initial hatching rate was lower. Larvae exposed at 2 and 26 days post hatching, exhibited a higher mortality rate than the corresponding non-exposed groups. In the other larval and juvenile stages, no short-term impact of exposure on survival was observed. Morphometric analysis of larvae and juveniles revealed no differences in measurements or deformations of the yolk, notochord, eye and head. In Chapter 5 sole embryos and larvae were investigated as a model for flatfish species with a specific metamorphosis during development. Exposure of sole embryos at 2 days post fertilisation and larvae at 11 days post hatching to pulsed direct current used to catch brown shrimp did not result in a lower survival eight days post exposure. Additionally, no differences in yolk sac resorption and morphometric length measurements of the notochord, muscle, eye and head, were observed in the developing larvae of exposed and control groups.

In Chapter 6 the role of pulsed direct current on the electro-detection ability of the small-spotted catshark, *Scyliorhinus canicula* was investigated. The **electroresponse of the sharks** to an artificially created prey-simulating electric field was tested before and after exposure to the pulsed electric field used to catch flatfish and shrimp. No statistically significant differences were noted between control and exposed animals, both in terms of the number of sharks exhibiting an electroresponse prior to and following exposure as well as regarding the timing between onset of searching behaviour and biting at the prey simulating dipole. These results indicate that, under the laboratory circumstances as adopted in this study, the small-spotted catshark is still able to detect the bioelectric field of a prey following exposure to pulsed direct current used in pulse trawls.

In the general discussion part (Chapter 7), the results of the different studies are summarized and discussed. Additionally, the need for further research is emphasized, outlining various research perspectives. Finally, possible problems considering pulse trawling for brown shrimp are highlighted.

To conclude, the direct impact of electrotrawling for brown shrimp seems rather small in comparison with traditional gear. However, gaps in knowledge and concerns still exist. Research regarding long term effects and other ecosystem components are recommended to ensure a sustainable brown shrimp fishery.

Acknowledgements –Dankwoord

Na een lange reis te hebben doorploeterd, zijn we eindelijk aan wal geraakt. Rest mij nog enkele belangrijke mensen te bedanken zonder wie ik hoogstwaarschijnlijk schipbreuk had geleden.

Bedankt, emer. Prof. dr. Magda Vincx, Dr. Jochen Depestele en Dr. Ir. Hans Polet om mij te begeleiden tijdens mijn eerste duik in de visserij wereld. Mijn Master thesis naar overleving van bijvangst in de boomkor visserij is waar het eigenlijk allemaal begon. Zonder de vele positieve ervaringen die ik daar mocht meemaken, was ik simpelweg nooit begonnen aan een doctoraat.

Verder ben ik onnoemelijk veel dank verschuldigd aan mijn promotoren Dr. Ir. Hans Polet, Prof. dr. Koen Chiers en in het bijzonder Prof. dr. Annemie Decostere. In de eerste plaats, bedankt om met mij in zee te willen gaan en ook onderweg in mij te blijven geloven. In moeilijke tijden boden jullie vaak een luisterend oor en stonden 7/7 klaar met advies en suggesties. Het aantal keren dat jullie dit manuscript hebben nagelezen is trouwens onmogelijk bij te houden. Gesteund door mijn promotoren verkreeg ik een onderzoeksbeurs van het Agentschap voor Innovatie door Wetenschap en Techniek (IWT) die mij de mogelijkheid gaf dit onderzoek te starten.

De Vakgroepen Morfologie en Pathologie, Bacteriologie en Pluimveeziekten van de Faculteit Diergeneeskunde van de Universiteit Gent, wil ik evenzeer bedanken, ondermeer Prof. dr. Paul Simoens voor zijn enthousiasme en alle laboranten voor de onschatbare hulp bij de verwerking van stalen en vele andere collega's voor de sfeer en gezelligheid. Door Marjan en het Museum Morfologie ging er een nieuwe wereld voor mij open. Autopsie en opgraven van potvissen, villen van tijgers, dissectie van haaien, maanvis en nog veel meer zijn stuk voor stuk unieke onvergetelijke ervaringen.

Naast de Faculteit Diergeneeskunde werd mijn tweede thuis het instituut voor Landbouw- en Vsserij Onderzoek (ILVO). Hier moet ik vooral de onderzoeksgroep "technisch visserij onderzoek" bedanken, in het bijzonder Fernand en Bart voor de infrastructuur, hulp en elektrisch advies bij het uitvoeren van proeven. Daarnaast ook Daan, David en Koenraad voor raad en daad bij de verzorging en kweek van de vele proefdieren.

Ook Wageningen Marine Research in Nederland droeg zijn steentje bij voor het opkweken van tong eitjes. Ook aan de onderzoeksgroep Evolutionary Morphology of Vertebrates van de vakgroep Biologie aan de Faculteit Wetenschappen van de Universiteit Gent, werd ik bijgestaan met apparatuur en waardevolle aanwijzingen bij de verdere verwerking van de vele stalen m.b.t jonge levensstadia.

Prof. dr. Luc Duchateau en Dr. Bart Ampe worden evenzeer bedankt voor de statistische kant van de zaak en uitleg die ik vaak tevergeefs probeerde te begrijpen.

De financiële steun van het Europees visserij Fonds (EVF) wordt bijzonder geapprecieerd.

Het neusje van de zalm was voor mij de proef die ik mocht uitvoeren op verschillende levensstadia van kabeljauw in het hoge Noorden van Noorwegen, ondersteund door Aquaexcel. Special thanks to Puvy and also Atle, Tove, Kjersti, Hannah and many other helpful hands at the Institute of Marine Research (IMR) and Centre for Marine Aquaculture Research (NOFIMA) for the hospitality, advice and support during my stay in Tromsø.

I also have to thank Stephen Kajiura, from Elsamolab, Florida, for the support regarding the shark experiment. My favourite A1 publication would not be there without borrowing me a prey simulator and all the valuable suggestions during the experimental set-up! Ook de Masterthesis studente Lisa Dumolein en haar hond Aiko hielpen me met veel enthousiasme bij dit experiment!

De bemanning van de onderzoeksschepen R.V. Simon Stevin (Flanders marine institute (VLIZ)), R.V. Belgica en de *Crangon* maar ook Marcel en Edwig worden oprecht bedankt voor de hulp in het verzamelen van proefdieren.

Tot slot bedank ik mijn ouders die altijd voor mij klaar staan en mij voor de volle 100% steunen, alsook Thomas voor het delen van lief en leed, Arthur voor mijn nieuwe blik op de werld en mij te leren relativeren en alle vrienden en vriendinnen die mij mijn gedachten konden doen verzetten…

About the Author

Marieke Desender was born on 28 May 1986. She finished her secondary school, Math and Economy, in 2004 in Beveren, Belgium. After finishing one year of Architecture studies in 2005 at Sint-Lucas, Ghent, she decided to change her study path. In 2005 she started studying Biology at Ghent University and graduated as a Master of Science in Biology cum laude in 2010 with a MSc thesis entitled "Mortality of discarded fish and invertebrates in beam trawl fisheries".

After graduation, she started teaching youngsters about physics and biology at the Koninklijk Lyceum Antwerpen (KLA) and Atheneum Gentbrugge in 2010-2011. Following, a PhD research grant from IWT was obtained in January 2012, regarding the impact of electrotrawling on various fish species in the North Sea. This research was carried out in cooperation with the Department of Morphology and Pathology, Bacteriology and Poultry Diseases at the Faculty of Veterinary Sciences (Ghent University) and the Institute of Agriculture and Fisheries Research (ILVO, Ostend). In 2012, a certificate as "proefleider" in Lab animal science lead was obtained. In 2013 she spent three months of training in Tromso, Norway to carry out experiments at the cod breeding centre (NOFIMA) and the Institute of Marine Research (IMR). She assisted in practical training of veterinary and aquaculture students in fish anatomy and pathology, respectively and was promotor of several Master thesises of Veterinarystudents. She is (co-)author of six international scientific publications in peer-reviewed journals. In addition, she actively participated in many (inter)national congresses. She received an early career scientist award at the ICES Annual Science conference 2012 in Bergen, Norway. She was an active member of ICES SG/WG ELECTRA since 2012. Currently she is working as a fisheries scientist at the Centre for Environment Fisheries and Aquaculture Science (CEFAS) in Lowestoft, UK.

Bibliography

Desender, M., Dumolein, L., Duchateau, L., Adriaens, D., Delbare D., Polet, H., Chiers, K., and Decostere, A. 2018. Electrotrawling: The impact of pulsed direct current on early life stages of sole (*Solea solea*). (In Press: North American Journal of Fisheries Management)

Desender, M., Decostere, A., Adriaens, D., Duchateau, L., Mortensen, A., Polet, H., Puvanendran, V., Verschueren, B. and Chiers, K. 2017b. Impact of pulsed direct current on embryonated eggs, larvae and young juveniles of Atlantic cod (*Gadus morhua*) and its implication in electrotrawling for brown shrimp. Marine and Coastal Fisheries 9; 330-340

Desender, M., Kajiura, S., Ampe, B., Dumolein, L., Polet, H., Chiers, K. and Decostere A. 2017a. Pulse trawling: evaluating its impact on prey detection by small-spotted catshark (*Scyliorhinus canicula*). Journal of Experimental Marine Biology and Ecology 486; 336-343

Desender, M., Chiers, K., Polet, H., Verschueren, B., Saunders, J., H., Ampe, B., Mortensen, A., Puvanendran, V., and Decostere, A., 2016. Short-term effect of pulsed direct current on various species of adult fish and its implication in pulse trawling for brown shrimp in the North Sea. Fisheries Research, 179: 90-97.

Uhlman, S., Theunynck, R., **Desender, M.**, Soetaert, M. and Depestele, J. 2016. Reflex impairment of beam-trawled flatfish. ICES Journal of Marine Science, Volume 73, Issue 4.

Depestele, J., **Desender, M**., Benoît, H. P., Polet, H. and Vincx M. 2014. Short-term survival of discarded target fish and non-target invertebrate species in the “eurocutter” beam trawl fishery of the southern North Sea. Fisheries Research, 154: 82-92.